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(54) **BORRELIDIN-DERIVATIVES AND THEIR USE IN MEDICINE**

BORRELIDIN-DERIVATE UND IHRE MEDIZINISCHE ANWENDUNG

DERIVES DE BORRELIDINE ET LEUR APPLICATION EN TANT QUE MEDICAMENTS

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to novel polyketides and uses thereof.

BACKGROUND TO THE INVENTION

[0002] Polyketides are natural products produced by a wide range of organisms, and particularly by microorganisms. Polyketides have many important pharmaceutical, veterinary and agricultural uses. Polyketides encompass a huge range of chemical structural space, and have a wide range of associated biological activities. Polyketides with use in medical treatments include antibiotics, immunosuppressants, antitumor agents, other chemotherapeutic agents, and other compounds possessing a broad range of therapeutic and biological properties. The Gram-positive bacteria *Streptomyces* and their allied genera are prodigious producers of polyketides, and the genetics and biochemistry of polyketide biosynthesis in these organisms are relatively well characterised (Hopwood, 1997). The genes for polyketide biosynthesis in *Streptomyces* are clustered and the exploitation of DNA technology has made it possible to isolate complete biosynthetic gene clusters by screening gene libraries with DNA probes encoding the genes responsible for their biosynthesis. Thus, increasing numbers of gene clusters for polyketide biosynthesis in *Streptomyces* and other microorganisms have been isolated and sequenced, including, for example, those for the polyether monensin (WO 01/68867), the polyene nystatin (WO 01/59126) and for rapamycin (Schwecke *et al.*, 1995).

[0003] Polyketides are synthesised through the repeated condensation of building blocks that contain a carboxylic acid function. At each stage of the process this results in the formation of a new β -keto function and an α -side chain branch into the growing chain. The structural diversity of polyketides derives from a number of aspects of their biosynthetic pathway including: the wide variety of starter units that may be utilised in their biosynthesis; the different lengths of polyketide chains that are possible; the various α -side chains that are introduced either during or after assembly of the polyketide chain; the various β -substitutions that may be introduced during or after assembly of the polyketide chain; the various degrees of processing that the β -keto groups can undergo (keto, hydroxyl, enoyl, and methylene); and the various stereochemistries that are possible at the α - and β -centres.

[0004] The synthesis of polyketides is catalysed by an enzyme, or by a complex of enzymes, called the polyketide synthase (PKS) in a manner similar to that of fatty acid biosynthesis. *Streptomyces* and related genera PKSs fall into three main categories: type-I, type-II and type-III. The type-III PKSs are small proteins related to plant chalcone synthases that have been discovered only recently (Moore & Hopke, 2000). Type-III systems have been implicated in the biosynthesis of a small number of secondary metabolites but may be more generally involved in the biosynthesis of soluble pigments (Cortes *et al.*, 2002). The type-II PKSs consist of several monofunctional proteins that act as a multi-polypeptide complex. Simple aromatic polyketides such as actinorhodin are formed by several rounds of chain assembly, which are performed iteratively on one set of type-II PKS enzymes that are encoded for by one set of PKS genes (Hopwood, 1997). Type-I PKSs are multifunctional proteins and are required for the synthesis of more complex polyketides such as erythromycin and rapamycin. As the focus of this patent, type-I PKS organisation and function are described in detail below:

[0005] Type-I PKSs are organised into modules, whereby each module consists of several catalytic 'domains' that are required to carry out one round of chain assembly (Staunton & Wilkinson, 1997). In general a modular PKS contains the correct number of modules (loading plus extension modules) to select and condense the correct number of loading and extension units. For example the erythromycin PKS consists of 7 modules (one loading and six extension modules) to select and condense the one starter and six extension units required for the biosynthesis of the erythromycin precursor 6-deoxyerythronolide B. Thus, there exists a one to one relationship between the number of modules present in the PKS and the number of units incorporated. This one to one relationship is described as 'co-linearity'.

[0006] The term 'extension module' as used herein refers to the set of contiguous domains, from the β -ketoacyl-acyl carrier protein synthase (KS) domain to the next acyl carrier protein (ACP) domain, which accomplishes one cycle of polyketide chain extension. The term 'loading module' as used herein refers to any group of contiguous domains that accomplishes the loading of the starter unit onto the PKS and thus renders it available to the KS domain of the first extension module. Besides condensation of the next extender carboxylic acid (or ketide) unit onto the growing polyketide chain, which is performed by the catalytic activity of the essential KS domain, modules of type-I PKSs may contain domains with β -ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER) activities which are responsible for the further processing of the newly formed β -keto groups during chain extension. The acyl transferase (AT) and the ACP domains present in each module are responsible for the choice of extender unit, and the tethering of the growing chain during its passage on the PKS respectively. The AT domains of a modular PKS can also be found as discrete proteins (Cheng *et al.*, 2003). The completed polyketide chain is generally released from PKSs by the action of a terminal thioesterase (TE) domain that is also generally involved in the cyclisation (lactonisation) of the final product. Other chain terminating/cyclising strategies are also employed such as that for the addition of an amino acid residue and macrolactam

formation as observed for rapamycin (Schwecke *et al.*, 1995), for macrolactam formation as for rifamycin (August *et al.*, 1998), and for amino acid incorporation followed by reductive elimination as for myxalamid biosynthesis (Silakowski *et al.*, 2001). In summary, there is a single enzymatic domain present for each successive catalytic step that occurs during biosynthesis on the PKS, and they are used in defined sequence that depends upon their location within the protein and the particular function they perform. This mechanism is termed 'processive'.

[0007] The modular arrangement of type-I PKSs was first confirmed by mutation of the erythromycin PKS (also known as 6-deoxyerythronolide B synthase, DEBS) through an in-frame deletion of a region of the KR domain of module 5 (Donadio *et al.*, 1991). This led to the production of the erythromycin analogues, 5,6-dideoxy-3- α -mycarosyl-5-oxoerythronolide B and 5,6-dideoxy-5-oxoerythronolide B, due to the inability of the mutated KR domain to reduce the β -keto group 5 at this stage of processive biosynthesis. Likewise, alteration of the active site residues in the ER domain of module 4 of DEBS2, by genetic engineering of the corresponding PKS-encoding DNA and its introduction into *Saccharopolyspora erythraea*, led to the production of 6,7-anhydroerythromycin C (Donadio *et al.*, 1993). In addition, the length of the polyketide chain formed by DEBS has been altered through the specific relocation of the TE domain of DEBS3 to the end of DEBS1; the expected triketide lactone product was produced in good yield (Cortés *et al.*, 1995). It should be noted that the changes described involved modification by deletion of sequence, or by sequence specific inactivation, or by the alternative juxtaposition of DNA sequence from within the same PKS cluster (ie. they are considered 'homologous changes'). Other such 'homologous' changes to the erythromycin PKS are described in WO 93/13663.

[0008] The modular organisation of type-I PKS genes lends itself to the manipulation of these genes to produce altered polyketide structures. Type I PKSs represent an assembly line for polyketide biosynthesis that can be manipulated by changing the number of modules; by changing their specificities towards different carboxylic acid starter units and extender units; by inactivating, mutating, removing, swapping or inserting domains with different activities and specificities; and by altering the chain or ring size through the repositioning of termination or cyclisation domains (Staunton & Wilkinson, 1997).

[0009] WO 98/01546 describes the production of hybrid PKS gene assemblies comprising the incorporation of *heterologous* DNA. WO 98/01546 describes methods for generating hybrid PKSs in which the substitution of genes encoding heterologous modules, sub-modules or domains for the native genes generates novel polyketides with altered structures. Specifically, for example the AT domains of heterologous DNA from the rapamycin or monensin PKSs can be exchanged for that native to the erythromycin PKS in order to generate novel polyketides with altered alkyl branching. Such an AT domain swap represented the first example of the production of a truly hybrid PKS (Oliynyk *et al.*, 1996). WO 98/01546 also describes in general terms the production of hybrid PKS assemblies comprising a loading module and at least one extension module. It specifically describes the construction of a hybrid PKS gene assembly by grafting the broad-specificity loading module for the avermectin-producing PKS onto the first protein of the erythromycin PKS (DEBS1) in place of the normal loading module (see also Marsden *et al.*, 1998). Additional examples comprising loading module swaps that are substrate specific have also been described (WO 00/00618; US 5876991; Kuhstoss *et al.*, 1996). WO 00/01827 describes methods for varying the β -keto processing capability of a PKS module through the ability to swap 'reductive loops', ie. the ability to rapidly and in a combinatorial manner, alter the number and type of ketoreductase, dehydratase and enoyl reductase domains within a module. In addition to changing the level of β -keto group processing, such changes may also lead to changes in stereochemistry of the α -alkyl and β -hydroxyl groups thus formed by the altered modules.

[0010] Although modular PKSs operate 'normally' in a co-linear and processive manner as described above, examples of a deviation from this mode of operation have been described and are discussed below.

[0011] The picromycin PKS gene cluster in *Streptomyces venezuelae* is responsible for the biosynthesis of both picromycin (a 14-membered, heptaketide macrolide) and methymycin (a 12-membered, hexaketide macrolide) (Xue *et al.*, 1998). The ability of a single PKS to produce two related macrolides, of different ring sizes, derives from the alternative expression of the final PKS gene *pikA4* (Xue & Sherman, 2000). When 'normal' expression occurs and full-length *PikA4* is formed, a sixth extension unit is incorporated and the picromycin aglycone is produced; when alternative expression occurs and an N-terminally truncated form of *PikA4* is produced, no sixth extension unit is incorporated and the growing polyketide chain is passed directly to the TE domain which leads to formation of the methymycin aglycone. Thus, a breakdown of co-linearity occurs and a 'ring contracted' product is formed. The biochemical basis for this phenomenon has been investigated and shown to be an ACP5 to ACP6 transfer, missing out covalent attachment to the intervening KS6 domain; such a breakdown of co-linearity has been called 'skipping' (Beck *et al.*, 2002).

[0012] Skipping has also been observed to occur when an extra extension module from the rapamycin PKS was interpolated into the erythromycin PKS in order to convert the natural heptaketide-producing PKS into an octaketide-producing one (Rowe *et al.*, 2001). The expected octaketide, 16-membered macrolide was produced, but the major product was the normal heptaketide product 6-deoxyerythronolide. This 'skipping' of the interpolated module is believed to occur due to the interpolated module acting on some occasions as a 'shuttle', passing the growing chain from the preceding module to the following downstream module without performing a round of chain extension. It was subsequently shown that the ACP domain of the interpolated module is essential in passing the growing polyketide chain from the

preceding ACP domain and passing it to the KS domain of the following module during skipping (Thomas *et al.*, 2002), a mechanism similar to that described for methymycin biosynthesis above. It is shown that skipping can occur without the active site nucleophile of the KS domain. A ring-contracted (skipped) nemadectin (an antiparasitic macrolide) has been reported from a mutant of a *Streptomyces* soil isolate that was modified by chemical mutation (Rudd *et al.*, 1990); the biosynthesis of the natural PKS product was abolished.

[0013] An alternative manner in which modular PKSs deviate from co-linear operation involves the iterative operation of modules. For example, module 4 of the erythromycin PKS appears to operate iteratively, at a low level, to produce a ring expanded 16-membered, octaketide macrolide related to 6-deoxyerythronolide B (Wilkinson *et al.*, 2000). The ability of the erythromycin PKS to perform this operation has been termed 'stuttering'. The 'stuttering' of the erythromycin PKS is considered an aberrant process, as the products of this stuttering are formed in low yield and the major product of the erythromycin PKS is the normal heptaketide 6-deoxyerythronolide B formed by co-linear operation. Products that appear to be formed by both stuttering and skipping have also been reported as minor components from the epothilone producer *Sorangium cellulosum* (Hardt *et al.*, 2001). The stigmatellin biosynthetic cluster of *Stigmatella aurantiaca* encodes for a PKS that comprises ten (one loading and nine extension) modules (Gaitatzis *et al.*, 2002); however, based on results from structural elucidation and the feeding of stable isotope labelled substrates, stigmatellin is formed from eleven modular derived units. Thus, it would appear that one of the stigmatellin PKS modules operates (twice) iteratively.

[0014] Since the priority filing of the present application, the sequence of the PKS responsible for biosynthesis of the macrolide lankacidin by *Streptomyces rochei* has been described (Mochizuki *et al.*, 2003). This PKS also appears to contain too few modules in comparison to the number of extension cycles required for lankacidin biosynthesis, although the mechanism by which this would occur is not clear.

[0015] Additional structural diversity can be generated through the modification of polyketides by enzymes other than the PKS, either during the process of chain assembly as seen during the biosynthesis of some ansamycins (Floss, 2001), or after the process of chain assembly following release from the PKS. Such non-PKS mediated reactions may include, but are not limited to the following: reduction, oxidation, hydroxylation, acylation, alkylation, amination, decarboxylation, dehydration, double bond isomerisation/migration, cyclisation, ring cleavage, conjugation, glycosylation, reductive elimination and any combination of these. When these reactions occur after chain assembly they are termed the post-PKS or tailoring steps. Such tailoring steps are generally, but not always, essential for endowing the polyketide natural product with biological activity.

[0016] In addition, the structural diversity of polyketides obtainable biosynthetically can be further enhanced through the use of defined heterologous post-PKS tailoring enzymes as well as through the use of those which naturally modify the natural polyketide (Gaisser *et al.*, 2000). WO 01/79520 describes the heterologous modification of polyketide macrolide structures through glycosylation, epoxidation, hydroxylation, and methylation. The ability to generate analogues of the agricultural compound spinosyn through glycosylation with alternative deoxyhexose substituents has been reported (Gaisser *et al.*, 2002).

[0017] Borrelidin 1 (Figure 1) is an 18-membered macrolide produced by several bacterial strains including, but not limited to, *Streptomyces rochei* ATCC23956, *Streptomyces parvulus* Tü113 and *Streptomyces parvulus* Tü4055. Borrelidin is herein shown to be derived from a *trans*-cyclopentane-1,2-dicarboxylic acid starter acid, three malonyl-CoA and five methylmalonyl-CoA extender units (see figure 2). From the absolute stereochemistry of borrelidin, based on the crystal structure and recently confirmed through total synthesis, the actual starter acid is predicted to be *trans*-cyclopentane-(1*R*,2*R*)-dicarboxylic acid. Borrelidin isolated after the feeding of stable isotope labelled acetate and propionate substrates clearly indicated the expected incorporation of these building blocks; in addition, it has been demonstrated in the present application that feeding of *trans*-cyclopentane-1,2-dicarboxylic acid was sufficient to re-establish borrelidin biosynthesis in mutants where specific genes believed to be involved in the formation of the starter unit had been disrupted. Borrelidin contains a nitrile group attached to the C12 position, which is shown herein to arise through the action of tailoring enzymes acting upon a methylmalonyl-CoA derived methyl branch present at this position. The gross structure of borrelidin was first elucidated in 1967 (Keller-Scheirlein, 1967), and was subsequently refined by detailed NMR analysis (Kuo *et al.*, 1989). The absolute configuration of borrelidin was confirmed by X-ray crystallography (Anderson *et al.*, 1989). Its co-identity as the antibiotic treponemycin has been verified (Maehr & Evans, 1987).

[0018] A number of groups have reported the synthesis of fragments of the borrelidin structure, and since the priority filing of the present application, two independent total syntheses of borrelidin have been reported (Hanessian *et al.*, 2003; Duffey *et al.*, 2003).

[0019] Borrelidin was first discovered due to its antibacterial activity (Berger *et al.*, 1949), although this antibacterial activity extends only to a limited number of micrococci, and is not found against all common test bacteria. The mode of action in sensitive microorganisms involves selective inhibition of threonyl tRNA synthetase (Paetz & Nass, 1973). Other activities against spirochetes of the genus *Treponema* (Singh *et al.*, 1985; US 4,759,928), against viruses (Dickinson *et al.*, 1965), uses for the control of animal pests and weeds (DE 3607287) and use as an agricultural fungicide (DE 19835669; US 6,193,964) have been reported. Additionally, since the priority filing of the present application, borrelidin has been reported to have antimalarial activity against drug resistant *Plasmodium falciparum* strains (Otoguro *et al.*,

2003). Between all of these reports only two reported any synthetically modified derivatives. The first of these describes the benzyl ester and its bis-O-(4-nitrobenzoyl) derivative (Berger *et al.*, 1949). The second of these describes the borrelidin methyl ester, the methyl ester bis O-acetyl derivative, and the methyl ester Δ_{14-15} -dihydro-, $\Delta_{14-15,12-13}$ -tetrahydro-, and $\Delta_{14-15,12-13}$ -tetrahydro-C12-amino derivatives (Anderton & Rickards, 1965). No biological activity was reported for any of these compounds.

[0020] A recent disclosure of particular interest is the discovery that borrelidin displays anti-angiogenesis activity (Wakabayashi *et al.*, 1997). Angiogenesis is the process of the formation of new blood vessels. Angiogenesis occurs only locally and transiently in adults, being involved in, for example, repair following local trauma and the female reproductive cycle. It has been established as a key component in several pathogenic processes including cancer, rheumatoid arthritis and diabetic retinopathy. Its importance in enabling tumours to grow beyond a diameter of 1-2 cm was established by Folkman (Folkman, 1986), and is provoked by the tumour responding to hypoxia. In its downstream consequences angiogenesis is mostly a host-derived process, thus inhibition of angiogenesis offers significant potential in the treatment of cancers, avoiding the hurdles of other anticancer therapeutic modalities such as the diversity of cancer types and drug resistance (Matter, 2001). It is of additional interest that recent publications have described the functional involvement of tyrosinyl- and tryptophanyl tRNA synthetases in the regulation of angiogenesis (Wakasugi *et al.*, 2002; Otani *et al.*, 2002).

[0021] In the rat aorta matrix culture model of angiogenesis, borrelidin exhibits a potent angiogenesis-inhibiting effect and also causes disruption of formed capillary tubes in a dose dependent manner by inducing apoptosis of the capillary-forming cells (Wakabayashi *et al.*, 1997). Borrelidin inhibited capillary tube formation with an IC_{50} value of 0.4 ng/ml (0.8 nM). In the same study, borrelidin was shown to possess anti-proliferative activity towards human umbilical vein endothelial cells (HUVEC) in a cell growth assay; the IC_{50} value was measured at 6 ng/ml, which is 15-fold weaker than the anti-angiogenesis activity measured in the same medium. This anti-proliferative activity of borrelidin was shown to be general towards various cell lines. In addition to these data the authors report that borrelidin inhibits tRNA synthetase and protein synthesis in the cultured rat cells; however the IC_{50} value for anti-angiogenesis activity (0.4 ng/ml) was 50-fold lower than that reported for inhibition of protein synthesis (20 ng/ml), indicating different activities of the compound.

[0022] Borrelidin also displays potent inhibition of angiogenesis *in vivo* using the mouse dorsal air sac model (Funahashi *et al.*, 1999), which examines VEGF-induced angiogenesis and is an excellent model for studying tumour-angiogenesis. Borrelidin was administered at a dose of 1.8 mg/kg by intraperitoneal injection and shown to significantly reduce the increment of vascular volume induced by WiDr cells, and to a higher degree than does TNP-470, which is a synthetic angiogenesis inhibitor in clinical trials. Detailed controls verified that these data are for angiogenesis inhibition and not inhibition of growth of the tumour cells. The authors also showed that borrelidin is effective for the inhibition of the formation of spontaneous lung metastases of B16-BL6 melanoma cells at the same dosage by inhibiting the angiogenic processes involved in their formation.

[0023] JP 9-227,549 and JP 8-173,167 confirm that borrelidin is effective against WiDr cell lines of human colon cancer, and also against PC-3 cell lines of human prostate cancer. JP 9-227,549 describes the production of borrelidin by *Streptomyces rochei* Mer-N7167 (Ferm P-14670) and its isolation from the resulting fermentation culture. In addition to borrelidin **1**, 12-desnitrile-12-carboxyl borrelidin **2** (presumably a biosynthetic intermediate or shunt metabolite), 10-desmethyl borrelidin **3** (presumably a biosynthetic analogue arising from the mis-incorporation of an alternative malonyl-CoA extender unit in module 4 of the borrelidin PKS), 11-epiborrelidin **4** and the C14,C15-cis borrelidin analogue **5** were described (see figure 1). Thus, JP 9-227,549 specifies borrelidin and borrelidin analogues wherein a nitrile or carboxyl group is attached the carbon skeleton at C12, and a hydrogen atom or lower alkyl group is attached to the carbon skeleton at C10.

[0024] WO 01/09113 discloses the preparation of borrelidin analogues that have undergone synthetic modification at the carboxylic acid moiety of the cyclopentane ring. The activity of these compounds was examined using endothelial cell proliferation and endothelial capillary formation assays in a similar manner to that described above. In general, modification of the carboxyl moiety improved the selectivity for inhibiting capillary formation: the major reason for this improvement in selectivity is through a decrease in the cell proliferation inhibition activity whereas the capillary formation inhibitory activity was altered to a much lower degree. Specifically, the borrelidin-morpholinoethyl ester showed a 60-fold selectivity index, the borrelidin-amide showed a 37-fold selectivity index, the borrelidin-(2-pyridyl)-ethyl ester showed a 7.5-fold selectivity index and the borrelidin-morpholinoethyl amide showed a 6-fold selectivity index, for the capillary formation inhibitory activity versus cell proliferation with respect to borrelidin. The capillary formation inhibitory activity of these and other borrelidin derivatives was verified using a micro-vessel formation assay. In addition, the authors showed that borrelidin weakly inhibited the propagation of metastatic nodules, after removal of the primary tumour, when using a Lewis lung adenocarcinoma model. However, the borrelidin-(3-picolylamide) derivative was reported to inhibit very considerably the increase of micrometastases in rats after intraperitoneal and also with *per os* administration at subtoxic doses. Similarly, using the colon 38 spleen liver model, the metastasis-forming ability of mouse colon adenocarcinoma cells transplanted into mouse spleen was considerably decreased after treatment with a subtoxic dose of this borrelidin derivative. These data confirm the earlier reported ability of borrelidin and its derivatives to inhibit the

formation of metastases.

[0025] Borrelidin has also been identified as an inhibitor of cyclin-dependant kinase Cdc28/Cln2 of *Saccharomyces cerevisiae* with an IC_{50} value of 12 $\mu\text{g/ml}$ (24 μM) (Tsuchiya *et al.*, 2001). It was shown that borrelidin arrests both haploid and diploid cells in late G_1 phase (at a time point indistinguishable from α -mating pheromone), and at concentrations that do not affect gross protein biosynthesis. These data were taken to indicate that borrelidin has potential as a lead compound to develop anti-tumour agents.

[0026] Since the priority filing of the present application, two further reports have been published concerning the biological activity of borrelidin. The first of these indicates that the anti-angiogenic effects of borrelidin are mediated through distinct pathways (Kawamura *et al.*, 2003). High concentrations of threonine were found to attenuate the ability of borrelidin to inhibit both capillary tube formation in the rat aorta culture model and HUVEC cells proliferation; however, it did not affect the ability of borrelidin to collapse formed capillary tubes or to induce apoptosis in HUVEC. Borrelidin was also found to activate caspase-3 and caspase-8, and inhibitors of both of these suppressed borrelidin induced apoptosis in HUVEC. The second of these papers used the method of global cellular mRNA profiling to provide insight into the effects of borrelidin on *Saccharomyces cerevisiae* (Eastwood and Schaus, 2003). This analysis showed the induction of amino acid biosynthetic enzymes in a time-dependent fashion upon treatment with borrelidin, and it was ascertained that the induction of this pathway involves the *GCN4* transcription factor.

[0027] In summary, the angiogenesis-inhibitory effect of borrelidin is directed towards the twin tumour-biological effects of proliferation and capillary formation. In addition, borrelidin, and derivatives thereof, have been shown to inhibit the propagation of metastases. Borrelidin also has indications for use in cell cycle modulation. Thus, borrelidin and related compounds are particularly attractive targets for investigation as therapeutic agents for the treatment of tumour tissues, either as single agents or for use as an adjunct to other therapies. In addition, they may be used for treating other diseases in which angiogenesis is implicated in the pathogenic process, including, but not restricted to, the following list: rheumatoid arthritis, psoriasis, atherosclerosis, diabetic retinopathy and various ophthalmic disorders.

SUMMARY OF THE INVENTION

[0028] The present disclosure provides the entire nucleic acid sequence of the biosynthetic gene cluster responsible for governing the synthesis of the polyketide macrolide borrelidin in *Streptomyces parvulus* Tü4055. Also provided is the use of all or part of the cloned DNA and the nucleic acid sequences thereof in the specific detection of other polyketide biosynthetic gene clusters, in the engineering of mutant strains of *Streptomyces parvulus* and other suitable host strains for the production of enhanced levels of borrelidin, or for the production of modified or novel polyketides, and of recombinant genes encoding PKS systems for the biosynthesis of modified or novel polyketides.

[0029] The complete nucleotide sequence of the borrelidin biosynthetic gene cluster from *Streptomyces parvulus* Tü4055 is shown in SEQ ID NO.1. Its organisation is presented in figure 3 and comprises genes and open reading frames designated hereinafter as: *borA1*, *borA2*, *borA3*, *borA4*, *borA5*, *borA6*, *borB*, *borC*, *borD*, *borE*, *borF*, *borG*, *borH*, *borI*, *borJ*, *borK*, *borL*, *borM*, *borN*, *borO*, *orfB1*, *orfB2*, *orfB3*, *orfB4*, *orfB5*, *orfB6*, *orfB7*, *orfB8*, *orfB9*, *orfB10*, *orfB11*, *orfB12*, *orfB13*, *orfB14*, *orfB15*, *orfB16*, *orfB17*, *orfB18*, *orfB19*, *orfB20*, *orfB21* and *orfB22*.

[0030] The proposed functions of the cloned genes are described in Figures 4 (proposed biosynthesis of the starter unit), 5 (organisation of the borrelidin PKS and biosynthesis of pre-borrelidin) and 6 (introduction of the C12-nitrile moiety) and are described below.

[0031] The present disclosure thus provides an isolated nucleic acid molecule comprising:

- (a) a nucleotide sequence as shown in SEQ ID No.1, or a portion or fragment thereof; or
- (b) a nucleotide sequence which is the complement of SEQ ID No.1, or a portion or fragment thereof; or
- (c) a nucleotide sequence which is degenerate with a coding sequence of SEQ ID No.1, or a portion or fragment thereof.

[0032] As used herein the term "fragment" with respect to nucleotide sequences refers to a stretch of nucleic acid residues that are at least 10, preferably at least 20, at least 30, at least 50, at least 75, at least 100, at least 150 or at least 200 nucleotides in length. A preferred portion or fragment of SEQ ID NO:1 is the sequence extending between nucleotide positions 7603 and 59966 of SEQ ID No.1.

[0033] The sequence may encode or be complementary to a sequence encoding a polypeptide of a polyketide biosynthetic gene cluster, or a portion thereof. By "a polypeptide of a polyketide biosynthetic gene cluster" is meant a polypeptide encoded by one or more open reading frames of a polyketide biosynthetic gene cluster, and particularly the borrelidin biosynthetic gene cluster.

[0034] A polyketide biosynthetic gene cluster is a segment of DNA comprising a plurality of genes encoding polypeptides having activity in the biosynthesis of a polyketide or macrolide moiety. This is not restricted to components of the polyketide synthase (PKS) which function *inter alia* in the synthesis of the polyketide backbone and reductive processing of side

groups, but also encompasses polypeptides having ancillary functions in the synthesis of the polyketide. Thus polypeptides of the biosynthetic gene cluster may also act in macrolide ring or polyketide chain modification (e.g. catalysing a reaction in the formation of the C12 nitrile moiety of borrelidin), in the synthesis of a precursor or starter unit for a polyketide or macrolide moiety (e.g. catalysing a reaction in the synthesis of the *trans*-cyclopentane-1,2-dicarboxylic acid starter unit for the borrelidin PKS, or responsible for the activation of such molecules as the coenzyme-A thioesters of the starter and extender units of the chain), regulatory activity (e.g. regulation of the expression of the genes or proteins involved in polyketide or macrolide synthesis), transporter activity (e.g. in transport of substrates for the polyketide or macrolide moiety into the cell, or of synthesis products such as the polyketide or macrolide molecule out of the cell), and in conferring resistance of the producing cell to the synthesised products (e.g. through specific binding to the synthesised molecule, or as a replacement for other endogenous proteins to which the synthesised molecule may bind within or outside of the cell).

[0035] The gene cluster also includes non-coding regions, such as promoters and other transcriptional regulatory sequences which are operably linked to the coding regions of the gene cluster. The skilled person is well able to identify such elements based upon the information provided herein, and these are within the scope of the present disclosure.

[0036] Genes and open reading frames encoded within SEQ ID No.1 represent preferred parts or fragments of SEQ ID No.1. Thus an isolated nucleic acid molecule may comprise a sequence that encodes a polypeptide from a borrelidin biosynthetic gene cluster, wherein said polypeptide has an amino acid sequence selected from the group consisting of SEQ ID Nos.2 to 43 and 113.

[0037] In preferred embodiments, the nucleic acid sequence comprises an open reading frame selected from the group of open reading frames of SEQ ID NO: 1 consisting of *borA1*, *borA2*, *borA3*, *borA4*, *borA5*, *borA6*, *borB*, *borC*, *borD*, *borE*, *borF*, *borG*, *borH*, *borI*, *borJ*, *borK*, *borL*, *borM*, *borN*, *borO*, *orfB1*, *orfB2*, *orfB3*, *orfB4*, *orfB5*, *orfB6*, *orfB7*, *orfB8*, *orfB9*, *orfB10*, *orfB11*, *orfB12*, *orfB13a*, *orfB13b*, *orfB14*, *orfB15*, *orfB16*, *orfB17*, *orfB18*, *orfB19*, *orfB20*, *orfB21* and *orfB22*, said open reading frames being described by, respectively, bases 16184*-18814, 18875-23590, 23686-34188, 34185*-39047, 39122*-45514, 45514-50742, 7603-8397c, 8397-9194c, 9244-9996c, 9993-11165c, 11162-11980c, 11992-13611 c, 13608-15659*c, 50739*-52019, 52113-53477, 53486-54466, 54506-56176, 56181*-57098, 57112-57858, 57939-59966, 2-313 (incomplete), 501*-3107, 3172-3810c, 3935-4924c, 5123-5953, 5961-6518*c, 6564*-7538, 60153-60533*c, 60620-61003, 61188*-61436, 61526-61738, 61767-62285c, 62750-63067c, 62586-62858c, 63155-65071c, 65374-65871, 65942-68305*c, 68290-68910*c, 69681-70436, 70445-71848, 71851-72957, 73037-73942 and 73995-74534c of SEQ ID No.1.

[0038] In the above list, 'c' indicates that the gene is encoded by the complementary strand to that shown in SEQ ID NO: 1. Each open reading frame above represents the longest probable open reading frame present. It is sometimes the case that more than one potential start codon can be identified. One skilled in the art will recognise this and be able to identify alternative possible start codons. Those genes which have more than one possible start codon are indicated with a '*' symbol. Throughout we have indicated what we believe to be the start codon, however, a person of skill in the art will appreciate that it may be possible to generate active protein using an alternative start codon, proteins generated using these alternative start codons are also considered within the scope of the present disclosure.

[0039] It should be noted that a number of these open reading frames begin with a codon (GTG, CTG or TTG) other than the more normal ATG initiation codon. It is well known that in some bacterial systems such codons, which normally denote valine (GTG) or leucine (CTG, TTG), may be read as initiation codons encoding methionine at the N terminus of the polypeptide chain. In the amino acid sequences (SEQ ID Nos: 2 to 43 and 113) provided herein, such codons are therefore translated as methionine.

[0040] Also provided are nucleic acid molecules comprising portions of the open reading frames identified herein. For example, such a nucleic acid sequence may comprise one or more isolated domains derived from the open reading frames identified herein. The polypeptides encoded by these isolated portions of the open reading frames may have independent activity, e.g. catalytic activity. In particular, the polypeptides which make up the borrelidin PKS have modular structures in which individual domains have particular catalytic activities as set out above. Thus any of these domains may be expressed alone or in combination, with other polypeptides from the borrelidin PKS described herein or domains thereof, or with polypeptides from the PKS of other polyketides. In particular, any of these domains may be substituted for the equivalent domains either within the borrelidin PKS or in other polyketide synthases and additionally equivalent domains from other PKSs may be substituted for domains within the borrelidin PKS. In this context an equivalent domain includes domains which have the same type of function but differ in for example, their specificity, an example of substitutions contemplated by the present disclosure include: the substitution of a malonyl-CoA specific AT domain for a methylmalonyl-CoA specific AT domain, or the substitute of a reductive loop containing a KR domain only for one containing KR, DH and ER. In preferred embodiments the expressed domains represent at least one PKS module as described below.

[0041] The term 'PKS domain' as used herein refers to a polypeptide sequence, capable of folding independently of the remainder of the PKS, and having a single distinct enzymatic activity or other function in polyketide or macrolide synthesis including, but not restricted to β -ketoacyl-acyl carrier protein synthase (KS), acyl carrier protein (ACP), acyl

transferase (AT), β -ketoreductase (KR), dehydratase (DH), enoyl reductase (ER) or terminal thioesterase (TE).

[0042] Accordingly, the disclosure further provides:

(a) an isolated nucleic acid molecule comprising a sequence that encodes a PKS domain selected from AT0 and ACP0, said domains being described by, respectively, amino acids 322-664 and 694-763 of SEQ ID No.2. In a preferred embodiment, the PKS domain comprises a sequence selected from the group consisting of bases 17147-18175 and 18263-18472 of SEQ ID No.1;

(b) an isolated nucleic acid molecule comprising a sequence that encodes a PKS domain selected from KS1, AT1, KR1 and ACP1, said domains being described by, respectively, amino acids 34-459, 557-885, 1136-1379 and 1419-1486 of SEQ ID No.3. In a preferred embodiment, the PKS domain comprises a sequence selected from the group consisting of bases 18974-20251, 20543-21529, 22280-23011 and 23129-23332 of SEQ ID No.1;

(c) an isolated nucleic acid molecule comprising a sequence that encodes a PKS domain selected from KS2, AT2, DH2, KR2, ACP2, KS3, AT3, DH3, KR3 and ACP3, said domains being described by, respectively, amino acids 34-459, 559-887, 903-1050, 1354-1597, 1628-1694, 1724-2149, 2245-2576, 2593-2734, 3060-3307 and 3340-3406 of SEQ ID No.4. In a preferred embodiment, the PKS domain comprises a sequence selected from the group consisting of bases 23785-25062, 25360-26346, 26392-26835, 27745-28476, 28567-28767, 28855-30132, 30418-31413, 31462-31887, 32863-33606 and 33703-33903 of SEQ ID No.1;

(d) an isolated nucleic acid molecule comprising a sequence that encodes a PKS domain selected from KS4, AT4, KR4 and ACP4, said domains being described by, respectively, amino acids 34-459, 555-886, 1179-1423 and 1459-1525 of SEQ ID No.5. In a preferred embodiment, the PKS domain comprises a sequence selected from the group consisting of bases 34284-35561, 35847-36842, 37719-38453 and 38559-38759 of SEQ ID No.1;

(e) an isolated nucleic acid molecule comprising a sequence that encodes a PKS domain selected from KS5, AT5, DH5, ER5, KR5 and ACP5, said domains being described by, respectively, amino acids 34-457, 553-888, 905-1046, 1401-1690, 1696-1942 and 1975-2041 of SEQ ID No.6. In a preferred embodiment, the PKS domain comprises a sequence selected from the group consisting of bases 39221-40492, 40778-41785, 41834-42259, 43322-44191, 44207-44947 and 45044-45244 of SEQ ID No.1;

(f) an isolated nucleic acid molecule comprising a sequence that encodes a PKS domain selected from KS6, AT6, KR6, ACP6 and TE, said domains being described by, respectively, amino acids 37-457, 555-883, 1101-1335, 1371-1437 and 1461-1708 of SEQ ID No.7. In a preferred embodiment, the PKS domain comprises a sequence selected from the group consisting of bases 45622-46884, 47176-48162, 48814-49518, 49624-49824 and 49894-50637 of SEQ ID No.1.

[0043] In another of its aspects the disclosure provides an isolated nucleic acid molecule comprising a sequence that encodes a PKS module, said module being selected from the group consisting of amino acids 322-763 of SEQ ID No.2, 34-1486 of SEQ ID No.3, 34-1694 of SEQ ID No.4, 1724-3406 of SEQ ID No.4, 34-1525 of SEQ ID No.5, 34-2041 of SEQ ID No.6 and 37-1437 or 1708 of SEQ ID No.7. In a preferred embodiment, the module comprises a sequence selected from the group consisting of bases 17147-18472, 18974-23332, 23785-28767, 28855-33903, 34284-38759, 39221-45244, 45622-49824 or 50637 of SEQ ID No.1.

[0044] The term 'module' as used herein refers to a single polypeptide comprising a plurality of PKS domains each having a single distinct enzymatic activity in polyketide or macrolide synthesis including, but not restricted to β -ketoacyl-acyl carrier protein synthase (KS), acyltransferase (AT), acyl carrier protein (ACP), β -ketoreductase (KR), dehydratase (DH), or enoyl reductase (ER) or terminal thioesterase (TE). An extension module typically comprises a KS, AT and ACP domain (although some modular PKSs may encode their AT domains as independent proteins). An extension module may further comprise one or more domains capable of reducing a beta-keto group to a hydroxyl, enoyl or methylene group (said group of domains are referred to herein as a "reductive loop"). Thus a module comprising a reductive loop typically contains a KR domain, KR and DH domains, or KR, DH and ER domains.

[0045] A PKS may further comprise a TE domain to perform chain termination and/or cyclisation of the final product, or alternatively it may contain another functionality known to perform a similar function such as that for the addition of an amino acid residue and macrolactam formation as observed for rapamycin (Schwecke et al., 1995), for macrolactam formation as for rifamycin (August et al., 1998), and for amino acid incorporation followed by reductive elimination as for myxalamid biosynthesis (Silakowski et al., 2001).

[0046] The sequences provided herein provide means with which to manipulate and/or to enhance polyketide synthesis. Thus there is provided a method of modifying a parent polyketide synthase, comprising expressing a domain from a borrelidin polyketide synthase or a derivative thereof as described herein in a host cell expressing said parent polyketide synthase, such that the domain is incorporated into said parent polyketide synthase. There is further provided a method of modifying a parent polyketide synthase, comprising introducing into a host cell a nucleic acid encoding a domain from a borrelidin polyketide synthase, or a derivative thereof, wherein the host cell contains nucleic acid encoding said parent polyketide synthase, such that, when expressed, the domain is incorporated into said parent polyketide synthase. The

borrelidin PKS domain may be inserted in addition to the native domains of the parent PKS, or may replace a native parent domain. Typically the parent PKS will be a Type I PKS.

[0047] The present disclosure further provides methods of modifying a parent borrelidin PKS. A donor domain (e.g. from a Type I PKS) may be expressed in a host cell expressing said parent borrelidin PKS. There is further provided a method of modifying a parent borrelidin polyketide synthase comprising introducing into a host cell a nucleic acid encoding a domain from a donor polyketide synthase, wherein the host cell contains nucleic acid encoding said parent borrelidin polyketide synthase, such that, when expressed, the domain is incorporated into said parent borrelidin polyketide synthase.

[0048] Additionally or alternatively, a domain of the parent PKS may be deleted or otherwise inactivated; e.g. a parent domain may simply be deleted, or be replaced by a domain from a donor PKS, or a domain from a donor PKS may be added to the parent. Where a domain is added or replaced, the donor domain may be derived from the parent synthase, or from a different synthase.

[0049] These methods may be used to enhance the biosynthesis of borrelidin, to produce new borrelidin derivatives or analogues, or other novel polyketide or macrolide structures. The number and nature of modules in the system may be altered to change the number and type of extender units recruited, and to change the various synthase, reductase and dehydratase activities that determine the structure of the polyketide chain. Such changes can be made by altering the order of the modules that comprise the PKS, by the duplication or removal of modules that comprise the PKS, by the introduction of modules from heterologous sources, or by some combination of these various approaches.

[0050] Thus domains or modules of the borrelidin PKS may be deleted, duplicated, or swapped with other domains or modules from the borrelidin PKS, or from PKS systems responsible for synthesis of other polyketides (heterologous PKS systems, particularly Type I PKS systems), which may be from different bacterial strains or species. Alternatively domains or modules from the borrelidin PKS may be introduced into heterologous PKS systems in order to produce novel polyketide or macrolides. Combinatorial modules may also be swapped between the borrelidin polyketide synthase and other polyketide synthases, these combinatorial modules extend between corresponding domains of two natural-type modules, e.g. from the AT of one module to the AT of the next.

[0051] For example, a particular extender module may be swapped for one having specificity for a different extender unit (as described e.g. in WO98/01571 and WO98/01546), or mutated to display specificity or selectivity for a different extender unit e.g. as described below. Additionally or alternatively, introduction, deletion, swapping or mutation of domains or modules, such as the KR, DH and ER domains responsible for the processing of a given β -keto moiety, may be used to alter the level of reductive processing of an extender unit during polyketide synthesis. Such changes may also lead to changes in stereochemistry of the α -alkyl and β -hydroxyl groups thus formed by altered modules. In a preferred embodiment the BorA5 module may be introduced into a parent PKS to provide iterative addition of extender units to a polyketide backbone, e.g. expanding the ring size of a macrolide polyketide relative to that naturally produced by the parent PKS.

[0052] The borrelidin loading module is the first PKS loading module to be identified having specificity for an alicyclic di-carboxylic acid starter unit. Thus this module or a derivative thereof may be used to introduce alicyclic starter units into heterologous polyketide synthases. This need not be restricted to use of *trans*-cyclopentane-1,2,-dicarboxylic acid normally used as the borrelidin starter unit. The borrelidin loading module is herein shown also to be capable of directing incorporation of other starter units including *trans*-cyclobutane-1,2-dicarboxylic acid, 2,3-methylsuccinic acid and 2-methylsuccinic acid. The borrelidin starter unit may also be modified in a borrelidin producing cell, or replaced by a heterologous loading module, to introduce alternative starter units into the borrelidin synthetic pathway.

[0053] The position of the loading module of the PKS may be chosen (e.g. by fusing it to a particular location within the PKS) in order to control the ring size of the resultant polyketide/macrolide molecules.

[0054] The AT domains that determine the carboxylic acid-CoA thioester extender units may be deleted, modified or replaced. The ACP domains may also be deleted, modified or replaced. In addition domains that are not normally present in the borrelidin PKS but which are found in other modular PKS and/or mixed PKS/NRPS systems may be inserted. Examples include, but are not limited to: O-methyl transferase domains, C-methyl transferase domains, epimerisation domains, monooxygenase domains and dehydrogenase domains, aminotransferase domains and non-ribosomal peptide synthetase domains.

[0055] Further, the thioesterase domain of the borrelidin PKS may be altered or repositioned (e.g. fused to a chosen location within the PKS) in order to change its specificity and/or in order to release polyketide/macrolide molecules with a chosen ring size. Alternatively, heterologous thioesterase domains may be inserted into the borrelidin PKS to produce molecules with altered ring size relative to the molecule normally produced by the parent PKS, or to produce a free acid.

[0056] In yet another alternative, the amino acid incorporating and macrolactam forming domains from mixed NRPS/PKS systems such as that for rapamycin, or for related systems such as for rifamycin biosynthesis and myxalamid biosynthesis, or modules from NRPS systems (such as those for bleomycin biosynthesis) may be inserted into the PKS to produce novel polyketide related molecules of mixed origin.

[0057] The open reading frames encoding the PKS described herein may also comprise portions encoding non-

enzymatically active portions which nevertheless have a functional role as scaffold regions which space and stabilise the enzymatically active domains and/or modules of the PKS at appropriate distances and orientations, and which may have recognition and docking functions that order the domains and modules of the PKS in the correct spatial arrangement. Thus the nucleic acid sequences of the present disclosure comprise sequences encoding such scaffold regions, either alone or in combination with sequences encoding domains or modules as described above. It will be appreciated that the various manipulations of PKS coding sequences described above may give rise to hybrid PKS genes or systems. Thus the present disclosure also provides nucleic acids encoding such hybrid PKS systems. The disclosure therefore provides a nucleic acid construct comprising at least one first nucleic acid portion encoding at least one domain of a borrelidin PKS and a second nucleic acid portion or portions encoding at least one type I PKS domain which is heterologous to said borrelidin PKS. In preferred embodiments the construct comprises a hybrid polyketide synthase gene, said gene encoding at least one domain of a borrelidin PKS and at least one type I PKS domain which is heterologous to said borrelidin PKS. Further preferred embodiments are as described above.

[0058] In a further aspect, the present disclosure provides an isolated nucleic acid molecule comprising a sequence encoding a polypeptide which catalyses a step in the synthesis of a starter unit or substrate for polyketide synthesis, preferably in the synthesis of the *trans*-cyclopentane-1,2,-dicarboxylic acid moiety used as a starter unit by the borrelidin PKS. The polypeptide may have activity as a dehydrogenase, 3-oxoacyl-ACP-reductase, cyclase, F420 dependent dehydrogenase, or 2-hydroxyhepta-2,4-diene-1,7-dioate isomerase. Preferably the polypeptide comprises the sequence encoded by one of the group of genes consisting of *borC*, *borD*, *borE*, *borF*, *borG*, *borH*, *borK*, *borL*, *borM* and *borN*, as shown in SEQ ID NO: 8, 9, 10, 12, 13, 14, 17, 18, 19 or 20.

[0059] These genes may be rendered deleted, disrupted, or otherwise inactivated in a borrelidin-producing cell in order to abolish borrelidin production. Cell lines resulting from such changes may be chemically complemented by the addition of exogenous carboxylic acids which may be incorporated in place of the natural starter unit. Thus, new borrelidin related molecules may be synthesised, which are initiated from the exogenously fed carboxylic acid. Such an approach is termed mutasynthesis. The genes responsible for *trans*-cyclopentane-1,2,-dicarboxylic acid synthesis may be introduced into a heterologous polyketide producer cell to allow that cell to synthesise the alicyclic dicarboxylic acid as a starter unit for its own PKS.

[0060] Thus the present disclosure further provides a method for the production of borrelidin and borrelidin analogues at improved titres, said method comprising disrupting *borG* in the host strain, fermenting the resulting cell line and feeding an exogenous carboxylic acid. In various preferred embodiments the exogenous carboxylic acid is *trans*-cyclopentane-1,2-dicarboxylic acid or the exogenous carboxylic acid is selected from the group consisting of *trans*-cyclobutane-1,2-dicarboxylic acid, 2,3-dimethyl succinic acid and 2-methylsuccinic acid and/or the method additionally comprises deleting, modifying or replacing one or more borrelidin biosynthetic genes, or borrelidin polyketide synthase domains or modules. A person of skill in the art is aware that polyketide synthases may also be expressed in heterologous hosts, therefore the present disclosure also contemplates a method for the production of higher titres of borrelidin and borrelidin analogues in a heterologous host, said method comprising transforming a host cell with the entire borrelidin gene cluster with the exception of *borG* or disrupting the *borG* gene in situ once the gene cluster has been transferred.

[0061] Alternatively, genes responsible for the synthesis of the starter unit may be over-expressed in order to improve the fermentation titres of borrelidin or borrelidin related molecules. Thus the present disclosure further provides a method for increasing the titre of borrelidin and borrelidin derivatives or borrelidin related molecules and their derivatives, said method comprising upregulating a borrelidin biosynthetic gene involved in production of the starter unit, said gene selected from the group consisting of *borC*, *borD*, *borE*, *borF*, *borH*, *borK*, *borL*, *borM* and *borN*, in a preferred embodiment the upregulated gene is *borE* or *borL*.

[0062] In another approach the genes responsible for the synthesis of the starter unit may be modified, or replaced by other synthetic genes directing the production of altered carboxylic acids, leading to the production of borrelidin related molecules. These techniques may be complemented by the modification of the loading module of the PKS as described above.

[0063] In a further aspect, the present disclosure provides an isolated nucleic acid molecule comprising a sequence encoding a polypeptide which catalyses a step in the modification of a side chain of a polyketide moiety, for example in the conversion of a methyl group to a nitrile moiety, e.g. at C12 of pre-borrelidin (**14**). The polypeptide may have activity as a cytochrome P450 oxidase, amino transferase, or NAD/quinone oxidoreductase. Preferably the polypeptide comprises the sequence encoded by one of the group of genes consisting of *borI*, *borJ*, and *borK* as shown in SEQ ID NO: 15, 16 or 17.

[0064] Various of these genes may be deleted/inactivated such that borrelidin-related molecules, or shunt metabolites thereof, accumulate which represent intermediate stages of the process that introduces the nitrile moiety. The addition of heterologous genes to such systems may allow alternative elaboration of any accumulated biosynthetic intermediates or shunt metabolites thereof. Alternatively, the genes may be mutated in order to alter their substrate specificity such that they function on alternative positions of pre-borrelidin molecules in order to provide borrelidin-related molecules. In addition, the genes responsible for formation of the nitrile group may be over-expressed in order to improve the ferment-

tation titres of borrelidin or borrelidin-related molecules.

[0065] Alternatively, one, some or all of these genes may be introduced into cells capable of producing other polyketides to provide for desired side chain processing of that polyketide, e.g. the introduction of a nitrile moiety. This opens up the possibility of specific biosynthetic introduction of nitrile moieties into polyketides, particularly at side chains derived from methylmalonyl-CoA or ethylmalonyl-CoA extender units. Purified enzymes (see below) may also be used to effect the conversion of polyketide side chains to nitrile moieties *in vitro*.

[0066] The nucleotide sequences of the disclosure may be portions of the sequence shown in SEQ ID NO: 1, or the complement thereof, or mutants, variants, derivatives or alleles of these sequences. The sequences may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a coding nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the redundancy of the genetic code. Thus, nucleic acid according to the present disclosure may include a sequence different from the sequence shown in SEQ ID NO: 1 yet encode a polypeptide with the same amino acid sequence. Preferably mutants, variants, derivatives or alleles of the sequences provided encode polypeptides having the same enzymatic activity as those described herein.

[0067] Where the sequence is a coding sequence, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequences shown in SEQ ID Nos: 2 to 43 and 113. Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant, derivative or allele of any of the sequences shown is further provided by the present disclosure. Such polypeptides are discussed below. Nucleic acid encoding such a polypeptide may show greater than about 60% identity with the coding sequence of SEQ ID NO: 1, greater than about 70% identity, greater than about 80% identity, or greater than about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity therewith. Percentage identity may be calculated using one of the programs such as BLAST or BestFit from within the Genetics Computer Group (GCG) Version 10 software package available from the University of Wisconsin, using default parameters.

[0068] In preferred embodiments, whether coding or non-coding, the nucleotide sequences of the disclosure are capable of hybridising specifically with at least a portion of the sequence of SEQ ID NO: 1 or the complement thereof.

[0069] For example, hybridizations may be performed, according to the method of Sambrook *et al.* (Sambrook *et al.*, 1989), using a hybridization solution comprising: 5X SSC, 5X Denhardt's reagent, 0.5-1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1 % SDS; (3) 30 minutes-1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65°C in 1X SSC and 1% SDS, changing the solution every 30 minutes.

[0070] One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology is (Sambrook *et al.*, 1989):

$$T_m = 81.5^{\circ}\text{C} + 16.6\text{Log} [\text{Na}^+] + 0.41(\% \text{ G+C}) - 0.63 (\% \text{ formamide}) - 600/\text{\#bp in duplex}$$

[0071] As an illustration of the above formula, using $[\text{Na}^+] = [0.368]$ and 50% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C. Such hybridisation would be considered substantially specific to the nucleic acid sequence of the present disclosure.

[0072] The nucleic acids of the present disclosure preferably comprise at least 15 contiguous nucleotides of SEQ ID NO: 1. They may comprise 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 150, 200, 300, 500 or more contiguous nucleotides of SEQ ID NO: 1.

[0073] The nucleic acids may be used e.g. as primers or probes for the identification of novel genes or other genetic elements, such as transcriptional regulatory sequences, from polyketide or macrolide biosynthetic gene clusters, e.g. sequences encoding enzymes of the PKS, or domains or modules thereof, enzymes involved in the biosynthesis of a starter unit, enzymes modifying side chains of polyketide moieties, transporters, resistance genes and regulatory molecules as described.

[0074] Thus the present disclosure provides a method of identifying a novel polyketide biosynthetic gene cluster, or a portion thereof, comprising hybridising a sample of target nucleic acid with a nucleic acid of the present disclosure capable of hybridising specifically to a nucleic acid having the sequence of SEQ ID NO: 1 or a portion thereof. The target nucleic acid may be any suitable nucleic acid, and is preferably bacterial genomic DNA.

[0075] Typically, the method further comprises the step of detecting hybridisation between the sample of nucleic acid and the nucleic acid of the disclosure. Hybridisation may be measured using any of a variety of techniques at the disposal of those skilled in the art. For instance, probes may be radioactively, fluorescently or enzymatically labelled. Other

methods not employing labelling of probe include amplification using PCR, RNAase cleavage and allele specific oligo-nucleotide probing.

[0076] A method may include hybridization of one or more (e.g. two) probes or primers to target nucleic acid. Where the nucleic acid is double-stranded DNA, hybridization will generally be preceded by denaturation to produce single-stranded DNA. The hybridization may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridization. A screening procedure, chosen from the many available to those skilled in the art, is used to identify successful hybridization events and isolated hybridized nucleic acid.

[0077] Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on, as described above.

[0078] An isolated nucleic acid molecule of the disclosure may be an isolated naturally occurring nucleic acid molecule (i.e. isolated or separated from the components with which it is normally found in nature) such as free or substantially free of nucleic acid flanking the gene in the bacterial genome, except possibly one or more regulatory sequence(s) for expression. Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA. Where nucleic acid according to the disclosure includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

[0079] The present disclosure further provides a vector comprising a nucleic acid according to the present disclosure. The vector is preferably an expression vector comprising a nucleic acid encoding a polypeptide of a polyketide biosynthetic gene cluster (preferably a borrelidin biosynthetic gene cluster), or a portion thereof, as described. Suitable vectors comprising nucleic acid for introduction into bacteria or eukaryotic host cells can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral eg "phage", or "phagemid", as appropriate. For further details see, for example, Sambrook *et al.*, 1989. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Short Protocols in Molecular Biology, Second Edition, Ausubel *et al.* Eds, John Wiley & Sons 1992. The disclosures of Sambrook *et al.* and Ausubel *et al.* are incorporated herein by reference.

[0080] In another of its aspects the present disclosure provides an isolated polypeptide encoded by a nucleic acid molecule of the disclosure as described herein. More particularly, there is provided an isolated polypeptide comprising an amino acid sequence as shown in any one or more of SEQ ID Nos.2 to 43 and 113 or a portion thereof. As set out above, these amino acid sequences represent translations of the longest possible open reading frames present in the sequence of SEQ ID NO: 1 and the complement thereof. The first amino acid is always shown as Met, regardless of whether the initiation codon is ATG, GTG, CTG or TTG.

[0081] As used herein the term "polypeptide(s)" includes peptides, polypeptides and proteins, these terms are used interchangeably unless otherwise specified.

[0082] A polypeptide which is an amino acid sequence variant, allele, derivative or mutant of any one of the amino acid sequences shown may exhibit at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the polypeptide of any one of the SEQ ID Nos.2 to 43 and 113, or with a portion thereof. Particular amino acid sequence variants may differ from those shown by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20, 20-30, 30-50, 50-100, 100-150, or more than 150 amino acids. Percentage identity may be calculated using one of the programs such as FASTA or BestFit from within the Genetics Computer Group (GCG) Version 10 software package available from the University of Wisconsin, using default parameters.

[0083] The present disclosure also includes active portions, fragments, and derivatives of the polypeptides of the disclosure.

[0084] An "active portion" means a peptide which is less than the full length polypeptide, but which retains at least some of its essential biological activity. For example, isolated domains or modules of the PKS as described above may be regarded as active portions of the PKS

[0085] A "fragment" means a stretch of amino acid residues of at least five, at least six, or at least seven contiguous amino acids, often at least eight or at least nine contiguous amino acids, typically at least 10, at least 13 contiguous amino acids and, most preferably, at least 20, at least 25, at least 30, at least 50, at least 75, at least 100 or more contiguous amino acids. Fragments of the sequence may comprise antigenic determinants or epitopes useful for raising antibodies to a portion of the relevant polypeptide. Thus the polypeptide need not comprise a complete sequence provided in any one of SEQ ID Nos 2 to 43 and 113, but may comprise a portion thereof having the desired activity, e.g. an isolated domain or module, such as those of the PKS described above. It should be noted that the terms part, portion and fragment are used interchangeably in this specification; no particular significance should be ascribed to the specific use of one of these terms in any particular context.

[0086] A "derivative" of a polypeptide of the disclosure or a fragment thereof means a polypeptide modified by varying

the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve insertion, addition, deletion or substitution of one, two, three, five or more amino acids, without fundamentally altering the essential activity of the wild type polypeptide.

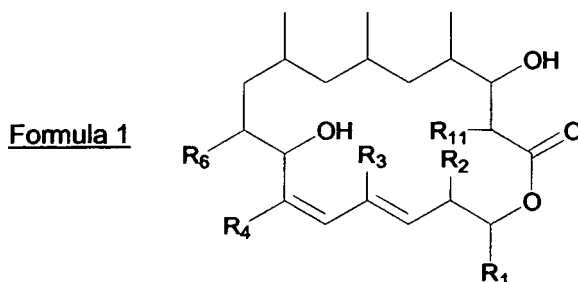
[0087] Polypeptides of the disclosure are provided in isolated form, e.g. isolated from one or more components with which they are normally found associated in nature. They may be isolated from a host in which they are naturally expressed, or may be synthetic or recombinant.

[0088] The present disclosure also encompasses a method of making a polypeptide (as disclosed), the method including expression from nucleic acid encoding the polypeptide (generally nucleic acid according to the disclosure). This may conveniently be achieved by growing a host cell in culture, containing an expression vector as described above, under appropriate conditions which cause or allow expression of the polypeptide. Polypeptides may also be expressed in *in vitro* systems, such as reticulocyte lysate systems.

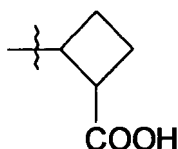
[0089] The method may include the step of introducing the nucleic acid into a host cell. The introduction, which may (particularly for *in vitro* introduction) be generally referred to without limitation as "transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, conjugation, electroporation and transfection using bacteriophage. As an alternative, direct injection of the nucleic acid could be employed. Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

[0090] Preferred host cells include Actinomycetes, preferably Streptomycetes, and in particular those selected from the group consisting of *Saccharopolyspora erythraea*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Micromonospora griseorubida*, *Streptomyces hygroscopicus*, *Streptomyces fradiae*, *Streptomyces longisporoflavus*, *Streptomyces lasaliensis*, *Streptomyces tsukubaensis*, *Streptomyces griseus*, *Streptomyces venezuelae*, *Streptomyces antibioticus*, *Streptomyces lividans*, *Streptomyces rimosus* and *Streptomyces albus*. *Streptomyces rochei* ATCC23956, *Streptomyces parvulus* Tü113 and *Streptomyces parvulus* Tü4055, more preferably selected from the group consisting of *Streptomyces rochei* ATCC23956, *Streptomyces parvulus* Tü113 and *Streptomyces parvulus* Tü4055.

[0091] In its principal aspect the invention provides for molecules that may be derived from the objects of the disclosure and for modified compounds formed therefrom and for methods for their production. The molecules derived from the objects of the disclosure are shown by formula 1 and extends to pharmaceutically acceptable salts thereof, wherein:



R₁ is a cycloalkyl group substituted as shown below;



wherein R₁ can also optionally be substituted with one or more halo atoms or one or more C₁ to C₃ alkyl groups; R₂, R₃, R₆, or R₁₁ are each independently H, OCH₃, CH₃ or CH₂CH₃; and R₄ is CN, CO₂H or CH₃. In preferred embodiments:

(a). R₄ is CH₃ or COOH

- (b). R₁ is cyclobutane-1'-carboxylate
 (c). R₆ is CH₃, R₂ and R₁₁ are H, and R₁ is cyclobutane-1'-carboxylate
 (d). R₁ is cyclobutane-1'-carboxylate, and R₄ is CH₃ or COOH.

[0092] The compounds of the present invention may have tRNA synthetase-inhibitory activity (e.g. they may inhibit threonyl-, tyrosinyl-, or tryptophanyl-tRNA synthetase). They may display anti-microbial activity, including activity against intra- or extracellular parasites and organisms such as bacteria, spirochetes (e.g. *Treponema*), malaria, viruses and fungi. Additionally or alternatively they may have anti-proliferative activity against mammalian cells, and/or anti-angiogenic activity, either as a result of tRNA synthetase inhibition, or through some other mode of action. This may make the compounds of the present invention particularly suitable as anti-cancer agents (e.g. agents for treatment of bowel cancer, prostate cancer or others), and may also provide application in treatment of other proliferative disorders, such as psoriasis, or conditions in which inappropriate vascularisation occurs, such as psoriasis, rheumatoid arthritis, atherosclerosis and diabetic retinopathy.

[0093] The compounds of the present invention may be formulated into pharmaceutically acceptable compositions, e.g. by admixture with a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such compositions also fall within the scope of the present invention.

[0094] Such pharmaceutically acceptable materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

[0095] Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

[0096] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringers Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

[0097] The invention further provides the compounds and compositions described above for use in a method of medical treatment. Also provided is the use of the compounds of the invention in the preparation of a medicament for the treatment of microbial conditions (including malaria), for the inhibition of angiogenesis, for the treatment of proliferative disorders, or for the treatment of conditions characterised by inappropriate vascularisation, as described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0098]

Figure 1 illustrates the structure of borrelidin and some related metabolites isolated from borrelidin producing organisms.

Figure 2 illustrates the incorporation patterns for ¹³C stable isotope labelled extension substrates and the position of the *trans*-cyclopentane-1,2-dicarboxylic acid starter unit derived carbons.

Figure 3 illustrates the organisation of the borrelidin biosynthetic gene cluster. Restriction sites: B, *Bam*HI; Bc, *Bcl*I; E, *Eco*RI; X, *Xho*I.

Figure 4 illustrates a scheme showing the proposed biosynthetic pathway for the *trans*-cyclopentane-1,2-dicarboxylic acid starter unit.

Figure 5 illustrates the organisation of the borrelidin PKS and the biosynthesis of the pre-borrelidin molecule.

Figure 6 illustrates the proposed biosynthetic route for the introduction of the nitrile moiety at the C12 position of borrelidin.

Figure 7 illustrates the proposed structure of the molecule **6**.

Figure 8 illustrates the proposed structure of the molecules **7** & **8**.

Figure 9 illustrates the molecular characterisation of the 4-hydroxyphenylacetic acid catabolic pathway in *E. coli* W.

Figure 10 illustrates the structures of the molecules **18-20**

Figure 11 illustrates the structures of the molecules **21-26**

DETAILED DESCRIPTION OF THE INVENTION

[0099] A cosmid library of *S. parvulus* Tü4055 genomic DNA was constructed using fragments obtained from a partial digestion with *Sau3A*I that were cloned into pWE15 and introduced into *E. coli* cells using the Gigapack® III Gold Packaging Extract kit (Stratagene). A library of 3000 *E. coli* transformants was screened for homology using a labelled probe that was generated using the DIG DNA Labelling and Detection Kit (Roche). The probe used was a 1.7 kbp *Bgl*II-*Bam*HI fragment obtained from the gene that encodes module 6 of the third subunit of the oleandomycin PKS from *Streptomyces antibioticus* (Swan *et al.*, 1994).

[0100] Clones that gave a positive response were selected and cosmid DNA isolated. Cosmid DNA was digested with *Bam*HI and fragments less than 3 kbp in size were sub-cloned into pOJ260 (Bierman *et al.*, 1992). The plasmids were then used to transform *S. parvulus* Tü4055 protoplasts and resulting mutants were screened for the ability to produce borrelidin. Two mutants were identified as borrelidin non-producers, both of which were derived from plasmids that contained fragments of cosBor32A2. These two fragments were of 1.97 and 2.80 kbp in size, and were later identified as adjacent fragments encoding parts of the borrelidin PKS (*borA2* & *borA3*). Using cosBor32A2 as the probe, a second overlapping cosmid, cosBor19B9 was identified from the original library. These two cosmids are sufficient to cover the entire borrelidin biosynthetic gene cluster (see figure 3).

[0101] The complete nucleotide sequence of cosBor32A2 and cosBor19B9 was determined by shotgun sequencing of a *Sau3A*I-derived subclone library for each cosmid, consisting of 1.5-2.0 kbp fragments in pHSG397 (Takeshita *et al.*, 1987). Specific details are provided in example 3. The complete, overlapping nucleotide-coding sequence for cosBor32A2 and cosBor19B9 is presented as SEQ ID No.1. The region encoded by cosmid cosBor32A2 represents the sequence from nucleotide positions 0-40217 bp of SEQ ID No.1. The region encoded by cosmid cosBor19B9 overlaps this region by 4452 nucleotides, and corresponds to the nucleotide positions 35766-74787 bp of SEQ ID No.1. As described in more detail in the following text, we have performed gene inactivation experiments on many of the orfs identified to be encoded within SEQ ID No.1, and this leads us to identify the limits of the cluster. The borrelidin biosynthetic gene cluster is contained between nucleotide positions 7603 to 59966 of SEQ ID No.1 (*borB* to *borO*, which includes the *borA* region). Thus, these combined efforts have led us to the identification and sequencing of the DNA region encompassing the entire borrelidin biosynthetic gene cluster, and to the identification and description of the functional sequences encoded within this region.

PKS GENES

[0102] Encoded between positions 16184-50742 of SEQ ID No.1 are 6 orfs that display very high homology to the genes that encode the PKSs of known macrolide producing organisms. These genes are designated *borA1*, *borA2*, *borA3*, *borA4*, *borA5* and *borA6*, and encode the borrelidin PKS as was demonstrated above by disruption of a 1.97 kbp region within *borA2*. The six orfs are arranged in a head-to-tail manner and each is terminated by an in-frame stop codon. The nucleotide sequence and corresponding polypeptide sequence details are shown below in Table 1:

Table 1

PKS encoding gene	Nucleotide position in SEQ ID No.1	Corresponding polypeptide sequence number
<i>borA1</i>	16184-18814	SEQ ID No.2
<i>borA2</i>	18875-23590	SEQ ID No.3
<i>borA3</i>	23686-34188	SEQ ID No.4
<i>borA4</i>	34185-39047	SEQ ID No.5
<i>borA5</i>	39122-45514	SEQ ID No.6
<i>borA6</i>	45514-50742	SEQ ID No.7

[0103] The gene *borA1* encodes the starter or loading module (SEQ ID No.1, position 16184-18814). The assignment of the start codon is not obvious for this open reading frame. The start codon given here is what we believe to be the true start codon, but there are at least another three possible start codons between the first and the beginning of the AT0 domain sequence and a person of skill in the art will appreciate that it may be possible to generate active protein using one of these alternative start codons. The start codon given here leaves a significant N-terminal tail of 321 amino acids preceding the AT0 domain. For comparison the N-terminal tail preceding the AT0 of the erythromycin loading module is 108 amino acids and that of the avermectin loading module is 28 amino acids. It is therefore possible that one of the other candidate start codons could be correct; the most likely of these are at positions 16298, 16607 and 16901

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of SEQ ID No.1. The length of the N-terminal tail suggests it could possibly represent a catalytic activity, although it does not have any significant homology to other sequences in the databases. The nucleotide sequence position and the corresponding amino acid sequence for each of the functional domains within the starter module are identified below in Table 2:

Table 2

Domain in <i>borA1</i>	Bases in SEQ ID No.1	Amino acids in SEQ ID No.2
AT0	17147-18175	322-664
ACP0	18263-18472	694-763

[0104] The gene *borA2* encodes the first extension module (SEQ ID No.1, position 18875-23590). The nucleotide sequence position and the corresponding amino acid sequence for each of the functional domains within the first extension module are identified below in Table 3:

Table 3

Domain in <i>borA2</i>	Bases in SEQ ID No.1	Amino acids in SEQ ID No.3
KS1	18974-20251	34-459
AT1	20543-21529	557-885
KR1	22280-23011	1136-1379
ACP1	23129-23332	1419-1486

[0105] The gene *borA3* encodes the second and third extension modules (SEQ ID No.1, position 23686-34188). The nucleotide sequence position and the corresponding amino acid sequence for each of the functional domains within the second and third extension modules are identified below in Table 4:

Table 4

Domain in <i>borA3</i>	Bases in SEQ ID No.1	Amino acids in SEQ ID No.4
KS2	23785-25062	34-459
AT2	25360-26346	559-887
DH2	26392-26835	903-1050
KR2	27745-28476	1354-1597
ACP2	28567-28767	1628-1694
KS3	28855-30132	1724-2149
AT3	30418-31413	2245-2576
DH3	31462-31887	2593-2734
KR3	32863-33606	3060-3307
ACP3	33703-33903	3340-3406

[0106] The gene *borA4* encodes the fourth extension module (SEQ ID No.1, position 34185-39047). The nucleotide sequence position and the corresponding amino acid sequence for each of the functional domains within the fourth extension module are identified below in Table 5:

Table 5

Domain in <i>borA4</i>	Bases in SEQ ID No.1	Amino acids in SEQ ID No.5
KS4	34284-35561	34-459
AT4	35847-36842	555-886

(continued)

Domain in <i>borA4</i>	Bases in SEQ ID No.1	Amino acids in SEQ ID No.5
KR4	37719-38453	1179-1423
ACP4	38559-38759	1459-1525

[0107] The gene *borA5* encodes the fifth extension module (SEQ ID No.1, position 39122-45514). The nucleotide sequence position and the corresponding amino acid sequence for each of the functional domains within the fifth extension module are identified below in Table 6:

Table 6

Domain in <i>borA5</i>	Bases in SEQ ID No.1	Amino acids in SEQ ID No.6
KS5	39221-40492	34-457
AT5	40778-41785	553-888
DH5	41834-42259	905-1046
ER5	43322-44191	1401-1690
KR5	44207-44947	1696-1942
ACP5	45044-45244	1975-2041

[0108] The gene *borA6* encodes the sixth extension module and the chain terminating thioesterase (SEQ ID No.1, position 45514-50742). The nucleotide sequence position and the corresponding amino acid sequence for each of the functional domains within the sixth extension module are identified below in Table 7:

Table 7

Domain in <i>borA6</i>	Bases in SEQ ID No.1	Amino acids in SEQ ID No.7
KS6	45622-46884	37-457
AT6	47176-48162	555-883
KR6	48814-49518	1101-1335
ACP6	49624-49824	1371-1437
TE	49894-50637	1461-1708

[0109] The identification of functional domains and their boundaries as described in the aforementioned are determined based on the similarities to the conserved amino acid sequences of other modular PKSs such as those for the rapamycin (Schwecke *et al.*, 1995; Aparicio *et al.*, 1996) and erythromycin (Cortés *et al.*, 1990) biosynthesis. The limits of the catalytic domains are established on the basis of homology to other PKS clusters and the chosen point at which a domain starts or finishes is not absolutely defined, but selected based on the aforementioned considerations. In the case of the β -keto processing domains it is least obvious, as there is typically a large region not assigned to a functional domain that precedes the KR domain. This region may be structurally important, or required for stability of the PKS dimer. An unusual characteristic of the borrelidin PKS is that all of the individual enzymatic domains appear to be catalytically competent based on their oligonucleotide/amino acid sequence, and are all necessary in order to provide the β -keto processing required to produce the functional groups observed in borrelidin. This is rather unusual as the majority of modular PKS sequences so far reported contain one or more inactive domains, an exception being for example the spinosyn PKS (Waldron *et al.*, 2001; US 6,274,50).

[0110] One skilled in the art is familiar with the degeneracy of the genetic code, therefore, the skilled artisan can modify the specific DNA sequences provided by this disclosure to provide proteins having the same or altered or improved characteristics compared to those polypeptides specifically provided herein. One skilled in the art can also modify the DNA sequences to express an identical polypeptide to those provided, albeit expressed at higher levels. Furthermore, one skilled in the art is familiar with means to prepare synthetically, either partially or in whole, DNA sequences which would be useful in preparing recombinant DNA vectors or coding sequences which are encompassed by the current disclosure. Additionally, recombinant means for modifying the DNA sequences provided may include for example site-

directed deletion or site-directed mutagenesis. These techniques are well known to those skilled in the art and need no further explanation here. Consequently, as used herein, DNA which is isolated from natural sources, prepared synthetically or semi-synthetically, or which is modified by recombinant DNA methods, is within the scope of the present disclosure.

[0111] Likewise, those skilled in the art will recognize that the polypeptides of the disclosure may be expressed recombinantly. Alternatively, those polypeptides may be synthesised either in whole or in part, by conventional known non-recombinant techniques; for example, solid phase synthesis. Thus, the present disclosure should not be construed as necessarily limited to any specific vector constructions or means for production of the specific biosynthetic cluster molecules including the polyketide synthase molecules exemplified.

[0112] The loading module of the borrelidin PKS exists as a discrete protein. This is rather unusual as the majority of loading modules are found on the same protein as the first extension module. Exceptions to this include, for example, the nystatin (Brautaset *et al.*, 2000) and amphotericin (Caffrey *et al.*, 2001) PKSs. The loading module, which consists of an AT-ACP didomain, is similar to the broad specificity loading module of the avermectin PKS, which accept a number of alternative starter adds, and are of use in generating libraries of novel polyketides (Marsden *et al.*, 1998; Pacey *et al.*, 1998). The AT domain of the borrelidin PKS loading module diverges from the vast majority of AT domains as the active site serine residue is replaced with a cysteine such that the active site motif is GXCXG (specifically GHCYG). In most available type-I PKS AT domain sequences, the conserved active site motif is GXSXG; the same motif is observed in lipases, fatty acid synthases and most thioesterases. The nucleophilic serine is substituted by cysteine in two NRPS thioesterase domains, specifically the synthetases responsible for the production of mycobactin and pyochelin (Shaw-Reid *et al.*, 1999). A GXCXG motif is also observed in a thioesterase-like domain of ORF1 in the bialaphos cluster (Raibaud *et al.*, 1991). It has been suggested that since it is not possible to move between the two types of serine codons by a single base change, active sites containing an essential serine residue may lie on two lines of descent from an ancient ancestral enzyme that had a cysteine instead of a serine in its active site (Brenner, 1988). The presence of enzymes containing cysteine in the active site may support this view. It may alternatively be the case that cysteine arises in these active sites because it is possible to move from one type of serine codon to the other via a cysteine which would remain active. The AT domains of PKSs select a particular carboxylic acid unit as substrate. This selectivity has been shown to correlate with certain motif signatures within the AT domain (Reeves *et al.*, 2001; WO 02/14482). The borrelidin loading module AT domain motif differs from any described so far, which is not surprising as this AT domain is the first to be sequenced that selects an alicyclic dicarboxylic acid. The AT domains for the borrelidin PKS extension modules display the expected active site motif GXSXG, and also each contain the expected motifs for the selection of malonyl-CoA or methylmalonyl-CoA (Reeves *et al.*, 2001; WO 02/14482). The malonyl-CoA selective AT domains (AT1, AT2 and AT6) show very high similarity to one another, both at the protein and at the DNA level. The same is true for the methylmalonyl-CoA selective AT domains (AT3, AT4 and AT5); two of these AT domains (AT3 and AT4) have identical amino acid sequences throughout the conserved region. The high similarity of AT5 to AT3 and AT4 is evidence that the extender unit selected in module 5 is methylmalonyl-CoA, and that the borrelidin C12-methyl group thus incorporated is subsequently modified to a nitrile function after incorporation into the PKS.

[0113] To demonstrate that we can alter the PKS derived structure of borrelidin, the AT domain of module 4 (the AT domain encoded by *borA4*) is replaced by the AT domain of module 2 of the rapamycin PKS (*rapAT2*) using a replacement strategy (see example 6). This gives strain *S. parvulus* Tü4055/467. Upon fermentation and LCMS analysis of culture extracts of this mutant, it can be determined that some borrelidin is produced and a new, more polar compound is also observed with a *m/z* value 14 units lower than borrelidin. This is consistent with incorporation of a malonate rather than a methylmalonate extender unit by module 4 of the PKS to produce 10-desmethyl borrelidin **3**. In addition to production by domain swapping methods, **3** is also generated by introducing specific mutations into the module 4 AT domain selectivity motif (Reeves *et al.*, 2001; WO 02/14482) (see example 7). Such a change affects the selectivity of the AT domain such that it selects a substrate molecule of malonyl-CoA preferentially over methylmalonyl-CoA. Thus, the amino acid motif YASH at positions 739 to 742 of SEQ ID No.5 is mutated to HAFH to give strain *S. parvulus* Tü4055/472. Upon fermentation and LCMS analysis of culture extracts of this mutant it is determined that borrelidin is produced in addition to a new, more polar compound with a *m/z* value 14 units lower than borrelidin. This new compound is identical to that described above and thus is consistent with incorporation of a malonate rather than a methylmalonate extender unit by module 4 of the PKS to produce **3**.

[0114] These results clearly indicate that the borrelidin PKS is amenable to genetic manipulation and to the exchange of native sequence for that of a heterologous strain. It is clear to one skilled in the art that the biosynthetic engineering, by the methods described above, of the borrelidin PKS will lead to the production of novel borrelidin-like molecules.

NON-PKS GENES

[0115] Both upstream and downstream of the PKS encoding genes are other orfs involved in the biosynthesis of borrelidin. An orf is designated as consisting of at least 100 contiguous nucleotides, that begins with an appropriate start

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codon and finishes with an appropriate stop codon, and which has an appropriate codon bias for protein-coding regions of an organism whose DNA is rich in the nucleotides guanine and cytosine. In the DNA sequence both upstream and downstream of the borrelidin PKS genes (*borA1-borA6*) there are a number of orfs that could be identified by comparison to other sequences in the NCBI database (see figure 3). The nucleotide sequence details of these orfs are given below in Table 8:

Table 8

Gene	Bases in SEQ ID No.1	Corresponding polypeptide sequence number
<i>borB</i>	7603-8397c	SEQ ID No.8
<i>borC</i>	8397-9194c	SEQ ID No.9
<i>borD</i>	9244-9996c	SEQ ID No.10
<i>borE</i>	9993-11165c	SEQ ID No.11
<i>borF</i>	11162-11980c	SEQ ID No.12
<i>borG</i>	11992-13611c	SEQ ID No.13
<i>borH</i>	13608-15599c*	SEQ ID No.14
<i>borI</i>	50739*-52019	SEQ ID No.15
<i>borJ</i>	52113-53477	SEQ ID No.16
<i>borK</i>	53486-54466	SEQ ID No.17
<i>borL</i>	54506-56176	SEQ ID No.18
<i>borM</i>	56181*-57098	SEQ ID No.19
<i>borN</i>	57112-57858	SEQ ID No.20
<i>borO</i>	57939-59966	SEQ ID No.21
<i>orfB1</i>	2-313	SEQ ID No.22
<i>orfB2</i>	501*-3107	SEQ ID No.23
<i>orfB3</i>	3172-3810c	SEQ ID No.24
<i>orfB4</i>	3935-4924c	SEQ ID No.25
<i>orfB5</i>	5123-5953	SEQ ID No.26
<i>orfB6</i>	5961-6518*c	SEQ ID No.27
<i>orfB7</i>	6564*-7538	SEQ ID No.28
<i>orfB8</i>	60153-60533c	SEQ ID No.29
<i>orfB9</i>	60620-61003	SEQ ID No.30
<i>orfB10</i>	61188*-61436	SEQ ID No.31
<i>orfB11</i>	61526-61738	SEQ ID No.32
<i>orfB12</i>	61767-62285c	SEQ ID No.33
<i>oriB13a</i>	62750-63067c	SEQ ID No.34
<i>orfB13b</i>	62586-62858c	SEQ ID No.113
<i>orfB14</i>	63155-65071c	SEQ ID No.35
<i>orfB15</i>	65374-65871	SEQ ID No.36
<i>orfB16</i>	65942-68305c*	SEQ ID No.37
<i>orfB17</i>	68290-68910c*	SEQ ID No.38
<i>orfB18</i>	69681-70436	SEQ ID No.39
<i>orfB19</i>	70445-71848	SEQ ID No.40

(continued)

Gene	Bases in SEQ ID No.1	Corresponding polypeptide sequence number
<i>orfB20</i>	71851-72957	SEQ ID No.41
<i>orfB21</i>	73037-73942	SEQ ID No.42
<i>orfB22</i>	73995-74534c	SEQ ID No.43
[Note 1: c indicates that the gene is encoded by the complement DNA strand; Note 2: for each open reading frame given above, the longest probable open reading frame is described. It is sometimes the case that more than one potential candidate start codon can be identified. One skilled in the art will recognise this and be able to identify alternative possible start codons. We have indicated those genes which have more than one possible start codon with a '*' symbol. Throughout we have indicated what we believe to be the start codon, however, a person of skill in the art will appreciate that it may be possible to generate active protein using an alternative start codon, proteins generated using these alternative start codons are also considered within the scope of the present disclosure. Note 3 the SEQ ID NO: for <i>orfB13b</i> was originally designated SEQ ID NO: 34 but for clarity a separate sequence and SEQ ID NO has been assigned.]		

[0116] Potential functions of the predicted polypeptides (SEQ ID No.7 to 43) were obtained from the NCBI database using a BLAST search. The best matches obtained from these searches are described below in Table 9:

Table 9

Gene	Significant protein match	Score	Accession GenBank	Proposed function
<i>orfB1</i>	hypothetical protein, no full length hits, high GC codon preference			unknown
<i>orfB2</i>	SCM2.07, hypothetical protein (<i>S. coelicolor</i>)	998	NP_625154	unknown
<i>orfB3</i>	SCF76.07, hypothetical protein, (<i>S. coelicolor</i>)	359	NP_624786	unknown
<i>orfB4</i>	SCF76.06, araC family transcriptional regulator (<i>S. coelicolor</i>)	412	NP_624785	unknown
<i>orfB5</i>	SCF76.05c, non-heme chloroperoxidase (<i>S. coelicolor</i>)	495	NP_624784	non-heme chloroperoxidase
<i>orfB6</i>	SCF76.09, hypothetical protein (<i>S. coelicolor</i>)	159	NP_624788	unknown
<i>orfB7</i>	SCF76.08c, hypothetical protein (<i>S. coelicolor</i>)	473	NP_624787	unknown
<i>borB</i>	PteH, polyene macrolide thioesterase (<i>S. avermitilis</i>)	244	BAB69315	type II thioesterase
<i>borC</i>	XF1726, 2,5-dichloro-2,5-cyclohexadiene-1,4,-diol dehydrogenase (<i>Xylella fastidiosa</i> strain 9a5c)e	160	NP_299015	dehydrogenase
<i>borD</i>	FabG, 3-oxoacyl-ACP reductase precursor, (<i>Plasmodium falciparum</i>)	124	AAK83686	3-oxoacyl-ACP reductase

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(continued)

Gene	Significant protein match	Score	Accession GenBank	Proposed function
<i>borE</i>	FN1586, O-succinylbenzoyl-CoA synthase, (<i>Fusobacterium nucleatum</i> subsp. <i>nucleatum</i> ATCC 25586)	88	NP_602402	cyclase (member of enolase superfamily)
<i>borF</i>	putative lysophospholipase homologue, (<i>Arabidopsis thaliana</i>)	57	NP_565066	unknown
<i>borG</i>	MTH1444, acetolactate synthase, large subunit, (<i>Methanothermobacter thermautotrophicus</i>)	120	NP_276558	Unknown
<i>borH</i>	PA3592, conserved hypothetical protein, (<i>Pseudomonas aeruginosa</i>)	116	NP_252282	unknown
<i>borI</i>	TylH1, cytochrome P450, (<i>Streptomyces fradiae</i>)	285	AAD12167	cytochrome P450 oxidase
<i>borJ</i>	BioA, DAPA aminotransferase, (<i>Kurthia</i> sp. 538-KA26)	346	BAB39453	amino transferase
<i>borK</i>	Adh1, alcohol dehydrogenase, (<i>Aquifex aeolicus</i>)	191	NP_213938	NAD/quinone oxidoreductase
<i>borL</i>	putative auxin-regulated protein GH3, (<i>Arabidopsis thaliana</i>)	92	NP_176159	unknown
<i>borM</i>	SCL6.10, hypothetical protein similar to putative F420-dependent dehydrogenase (<i>S. coelicolor</i>),	108	CAB76875	F420 dependent dehydrogenase
<i>borN</i>	SC1C2.27, hypothetical protein, 2-hydroxyhepta-2,4-diene-1,7-dioate isomerase superfamily (<i>S. coelicolor</i>)	215	NP_629680	2-hydroxyhepta-2,4-diene-1,7-dioate isomerase
<i>borO</i>	ThrS, threonyl-tRNA synthetase (<i>Mycobacterium leprae</i>)	627	NP_301410	threonyl-tRNA synthetase, self resistance gene
<i>orfB8</i>	conserved hypothetical protein (<i>Methanosarcina acetivorans</i> str. C2A). (Pfam pulls out weak MarR family)	37	NP_617908	possible regulator
<i>orfB9</i>	putative anti-sigma factor antagonist (<i>Streptomyces coelicolor</i>)	113	NP_631789	anti-sigma factor antagonist
<i>orfB10</i>	conserved hypothetical protein (<i>S. coelicolor</i>)	95	NP_631790	unknown
<i>orfB11</i>	hypothetical protein, no full length hits, high GC codon preference			unknown
<i>orfB12</i>	putative regulator (<i>S. coelicolor</i>)	92	NP_631494	regulator (of a two component system, maybe membrane sensor)

(continued)

Gene	Significant protein match	Score	Accession GenBank	Proposed function
<i>orfB13a</i>	putative acetyltransferase (<i>S. coelicolor</i>);	58	NP_625155	tentative assignment of acetyltransferase
<i>orfB13b</i>	putative acetyltransferase (<i>S. coelicolor</i>)	100	NP_625155	in two frames, or sequencing error and should be in a single frame
<i>orfB14</i>	putative lipoprotein (<i>S. coelicolor</i>)	386	NP_631245	unknown
<i>orfB15</i>	hypothetical protein (<i>S. coelicolor</i>)	41	NP_631424	unknown
<i>orfB16</i>	putative formate dehydrogenase (<i>S. coelicolor</i>) (Pfam matches to molybdopterin oxidoreductase/ formate dehydrogenase alpha subunit)	915	NP_626265	oxidoreductase
<i>orfB17</i>	conserved hypothetical protein, <i>S. coelicolor</i> SCBAC25F8.16	175	NP_631569	unknown
<i>orfB18</i>	product unknown (<i>Streptomyces aureofaciens</i>)	396	AAD23399	unknown
<i>orfB19</i>	putative aldehyde dehydrogenase (<i>S. aureofaciens</i>)	635	AAD23400	aldehyde dehydrogenase
<i>orfB20</i>	putative alcohol dehydrogenase (<i>S. coelicolor</i>)	450	NP_630527	alcohol dehydrogenase
<i>orfB21</i>	hypothetical protein (<i>S. coelicolor</i>)	395	NP_630528	unknown
<i>orfB22</i>	putative calcium binding protein (<i>S. coelicolor</i>)	160	NP_631687	calcium binding protein

[0117] Analysis of the functions of the putative gene products indicates that the genes *borB* to *borO* most probably form the boundaries of the borrelidin biosynthetic cluster. Evidence to support this came from the disruption of *borB2*, which produced borrelidin at levels indistinguishable from the wild type parental strain. In addition, *borB3* to *borB7* have homologues in the *Streptomyces coelicolor* A3(2) genome encoded on cosmid SCF76; the same orfs are present, but in a different order. The orfs *borB8* to *borB10* are arranged identically to homologues in the *S. coelicolor* A3(2) cosmid SC5E3. The orfs *borB18* to *borB21* have homologues that are arranged similarly in the *S. coelicolor* A3(2) cosmid SC1A2. The orf *borB13* contains a frame-shift and thus any gene product would most probably be inactive. In addition, no function can be readily deduced for the products of these orfs during borrelidin biosynthesis.

Starter unit biosynthesis genes

[0118] In order to identify the genes that are involved in the biosynthesis of the *trans*-cyclopentane-1,2-dicarboxylic starter unit, each of the genes *borB* to *borN* was disrupted (e.g. see examples 8-11). This was done in a manner designed to minimise the possibility of polar effects, which was verified by successful *in trans* complementation with a full-length copy of the disrupted gene under the control of the ermE* promoter, which gave back approximately WT levels of borrelidin production in each case.

[0119] Each of the disrupted mutants was grown in triplicate as described in example 1, and borrelidin production assessed. Alongside these, each mutant was grown in triplicate and supplemented, after 24 hours, with exogenous starter acid to a final concentration of 1 mM, and borrelidin production assessed. Extraction and analysis for borrelidin provided the data that are described below in Table 10:

Table 10

Borrelidin biosynthetic gene disrupted	Borrelidin production without feeding (%relative to WT)	Borrelidin production with feeding (%relative to unfed WT)
Wild type (control)	100±16, (100±2)	363±65, (269±49)
<i>borB</i>	75±11, (43±20)	172±51
<i>borC</i>	0, (10±3)	933±42
<i>borD</i>	7±1, (0)	75±15
<i>borE</i>	2±1	122±23
<i>borF</i>	3±2	201±52
<i>borG</i>	11±1, (32±3)	1532±142
<i>borH</i>	17±2, (23±13)	203±40
<i>borI</i>	0, (0)	0, (0)
<i>borJ</i>	0, (0)	0, (0)
<i>borK</i>	0, (6±1)	319±54, (464±18)
<i>borL</i>	0, (0)	408±70, (399±69)
<i>borM</i>	0, (6±3)	461±29, (553±66)
<i>borN</i>	25±9, (34±3)	68±12, (46±9)
<i>borO</i>	N/A	N/A
[Note 1: The values given in brackets indicate where repeat runs of some experiments were performed; Note 2: N/A = not applicable.]		

[0120] Based on the data in table 10, it is clear to one skilled in the art that the gene products BorC-F and K-M are essential or very important for the biosynthesis of *trans*-cyclopentane-1,2-dicarboxylic acid, as these mutants produced no or very low levels of borrelidin without the addition of exogenous starter acid, whereupon they produced borrelidin at levels approaching, or better than, that of the WT organism. In addition the gene products BorG, H, and N appear to be involved in, but not essential for, the biosynthesis of the starter unit, as they produced significantly lower levels of borrelidin unless exogenous starter acid was added, whereupon they produced borrelidin at levels approaching or better than that of the WT organism; this was particularly notable in the case of the *borG*⁻ mutant.

[0121] The normal metabolic function of BorN homologues is the production of 2-oxohepta-3-ene-1,7-dioate **10**, a key step in the catabolism of tyrosine *via* 4-hydroxyphenyl acetic acid **9** (figure 9) (Prieto *et al.*, 1996). Therefore, **10** may be an intermediate in the biosynthetic pathway to *trans*-cyclopentane-1,2-dicarboxylic. The ability of the mutant disrupted in *borN* to produce borrelidin, albeit at a reduced level, most probably lies in the presence of a homologue elsewhere in the genome utilised in the catabolism of tyrosine during primary metabolism.

[0122] The intermediate **10** contains all the required functionality for the eventual formation of *trans*-cyclopentane-1,2-dicarboxylic acid. The most probable next step of the biosynthesis is the reduction of the 3-ene position in a reaction similar to that catalysed by an enoyl reductase. Potential enzymes responsible for this step are BorC, BorD, BorK or BorM; these enzymes are all involved in borrelidin starter unit biosynthesis as seen from the data in table 10. The resulting 2-oxohepta-1,7-dioate **11** is one possible substrate for cyclisation through formation of a new C-C bond between C6 and C2. Another possible substrate for this cyclisation would be 2-hydroxyhepta-1,7-dioate **12** or some activated form thereof. This would presumably be formed from **11** by the action of an oxidoreductase such as BorC, BorD or BorM.

[0123] The key cyclisation step is most probably catalysed by BorE, which displays similarity to O-succinylbenzoyl-CoA synthase and chloromuconate cycloisomerase. These enzymes belong to the enolase super-family, the members of which share the common ability to stabilise the formation of an anion on the carbon atom adjacent to a carboxylate group (Schmidt. *et al.*, 2001). It is further notable that the substrate for muconate cycloisomerase is a hexa-1,6-dioate, which is similar in gross structure to **11** and **12**. Abstraction of a proton and formation of an anion equivalent at C6 of **11** or **12** (or an activated form thereof, e.g. **13**) with subsequent cyclisation to C2 provides the correctly substituted cyclopentane ring structure, although the intermediacy of **11** as substrate would require some further processing of the substituted cyclopentane, most probably via elimination of water to give the symmetric cyclopent-1-ene-1,2-dicarboxylic acid, or possibly the Δ^1 -unsaturated compound, cyclopent-1-ene-1,2-dicarboxylic acid. However, the feeding of cyclopent-1-ene-1,2-dicarboxylic acid, or ethyl esters thereof, to *S. parvulus* Tü4055 strains disrupted in any of *borC-E*,

or to WT strains, did not produce any borrelidin, or did not produce borrelidin in any increased amount when compared to the unfed controls. These data indicate that this compound is probably not an intermediate in starter unit biosynthesis, and that the substrate of BorE is possibly the 2-hydroxyhepta-1,7-dioate **12**, or an activated form thereof (e.g. **13**). A putative pathway for the biosynthetic pathway to *trans*-cyclopentane-1,2-dicarboxylic acid is shown in figure 4.

[0124] The combined, specific genes required for the biosynthetic steps to *trans*-cyclopentane-1,2-dicarboxylic acid are not clear, but probably are encoded by some combination of *borC-H*, *borK*, *borM* and *borN*. The lack of certain homologues of genes that are involved in the catabolism of 4-hydroxyphenyl acetic acid **9**, and which would act prior to BorN in the pathway, is most probably an indication that primary metabolic genes perform these tasks. The addition of exogenous *trans*-cyclopentane-1,2-dicarboxylic acid to *S. parvulus* Tü4055 and related strains increases the titre of borrelidin in the order of 2- to 3-fold under our conditions, indicating that the biosynthesis of starter acid is a limiting factor in borrelidin biosynthesis. These data are consistent with primary metabolic degradation of tyrosine being the source of *trans*-cyclopentane-1,2-dicarboxylic acid.

[0125] In an attempt to further clarify which genes may be specifically responsible for biosynthesis of the starter unit, a number of co-culture experiments were performed with combinations of the different mutants - these require the knowledge that the gene products of *borI* and *borJ* are specifically involved in the formation of the C12-nitrile moiety, which is clarified by the data given in the following section below in combination with the data from table 10. In summary, the co-culture of mutants *borE*⁻ & *borD*⁻, and of *borE*⁻ & *borM*⁻ failed to produce any borrelidin whereas the co-culture of mutants *borM*⁻ & *borI*⁻, and *borM*⁻ & *borK*⁻ produced borrelidin at approximately WT levels. These data, in combination with that in table 10, and below, clearly indicate that *borD*, *borE* and *borM* are involved in starter unit biosynthesis, whereas *borI*, and possibly *borK*, are involved in the formation of the nitrile moiety at C12 of borrelidin.

[0126] It is clear from the data in table 10 that exogenous addition of *trans*-cyclopentane-1,2-dicarboxylic acid is sufficient to re-establish approximately WT levels, or better, of borrelidin production in mutants where genes that are involved in starter unit biosynthesis have been disrupted. These data indicate that there is no problem with the active uptake of added carboxylic acid by *S. parvulus* Tü4055, and that an activity is present which is capable of converting the carboxylic acid to a CoA thioester equivalent. Thus, given the known technologies of mutasynthesis, it is obvious to one skilled in the art that the addition of exogenous carboxylic acids to one of the aforementioned mutants, for example the *borE*⁻ strain *S. parvulus* Tü4055/*borE::aac3(IV)* described in example 8, may lead to the production of borrelidin analogues in which the starter unit carboxylic acid moiety is replaced with a moiety derived from the exogenously added carboxylic acid.

[0127] To examine this possibility, strain *S. parvulus* Tü4055/*borE::aac3(IV)* was fed with a *trans*-cyclobutane-1,2-dicarboxylic acid according to the protocol described in example 1 and then analysed as described in example 4. The structure **18**, described in figure 10, shows the new borrelidin structure obtained from feeding this carboxylic acid; this compound **18** displayed the anticipated UV chromophore for borrelidin but eluted at an earlier retention time and displayed the expected mass by LCMS ($m/z = 474.3$ [M-H]⁻). Verification of this methodology was provided by the production, isolation and characterisation of **18** (example 16). It is clear to one skilled in the art that other carboxylic acids could also be used in similar feeding experiments to provide further new borrelidin analogues. Although it is possible that not all carboxylic acids would be incorporated using the exact methodology described herein, a person of skill in the art is aware of a number of available methods to enhance the incorporation of fed starter units.

[0128] In addition to the use of the strain deleted in *borE*, it was observed (see table 10) that the strain *S. parvulus* Tü4055/*borG::aac3(IV)*, in which *borG* has been disrupted, when fed with the natural starter unit of the bor PKS, *trans*-cyclopentane-1,2-dicarboxylic acid, produced borrelidin at titres significantly higher than those seen when the wild-type organism was fed (4-fold increase) or unfed (15-fold increase). To examine this further, this experiment was repeated using both the natural and an unnatural starter acid as exogenous substrates, fed, in parallel, to wildtype, the *borE* mutant and the *borG* mutant. The resulting data are described in table 11.

Table 11:

	Unfed	Fed with 1 mM cyclopentane <i>trans</i> -1,2-dicarboxylic acid	Fed with 1 mM cyclobutane <i>trans</i> -1,2-dicarboxylic acid
<i>S. parvulus</i> Tü4055	2.3 mg/l	6.6 mg/l	-
<i>S. parvulus</i> Tü4055/ <i>borE::aac3(IV)</i>	0	4.7 mg/l	2.2 mg/l
<i>S. parvulus</i> Tü4055/ <i>borG::aac3(IV)</i>	0	88.9 mg/l	43.0 mg/l

As one can see from table 11, using *S. parvulus* Tü4055/*borG::aac3(IV)* instead of *S. parvulus* Tü4055/*borE::aac3(IV)*

for mutasynthesis increases the titre approximately 19-fold, and that *S. parvulus* Tü4055/*borG::aac3(IV)* fed with the natural starter acid produces 38-fold more borrelidin A than wild type alone, or 13 fold more borrelidin A than the wild type strain fed with the same amount of cyclopentane *trans*-1,2-dicarboxylic acid. These data clearly indicate that the use of strain *S. parvulus* Tü4055/*borG::aac3(IV)* for mutasynthesis experiments is beneficial for the production of improved titres of borrelidin analogues. This method has general applicability for both the production of borrelidin and borrelidin analogues.

[0129] On the basis of this finding, the feeding experiments with alternative carboxylic acids were repeated in *S. parvulus* Tü4055/*borG::aac3(IV)*, and extended to include 2,3-dimethyl succinic acid and 2-methylsuccinic acid; the new compounds derived from the incorporation of these alternative starter units, **19** and **20** respectively, are described in figure 10.

Formation of the nitrile moiety at C12

[0130] Sequence analysis of the AT domain of the borrelidin PKS module 3 indicates that the substrate utilised for the third round of chain extension is methylmalonyl-CoA. Thus, the carbon atom of the nitrile moiety most probably arises from the methyl group of methylmalonyl-CoA. This was verified by stable isotope feeding experiments. Feeding [2,3-¹³C₂] sodium propionate to *S. parvulus* Tü113 gave borrelidin which displayed intact labelling of the carbons at C4-C24, C6-C25, C8-C26, C10-C27 and C12-C28, and identical specific incorporations (as determined within the limits of our experimental methods), as expected (figure 2). These data indicate that the conversion of the C12-methyl group occurs either during chain assembly at, or after, the incorporation of the third extension unit, or that it occurs after polyketide chain assembly and release from the PKS. Based on functional assignments given to the borrelidin biosynthetic genes, in conjunction with the gene disruption data described in table 10, both *borI* and *borJ* are clearly implicated in formation of the nitrile moiety at C12, while others such as *borK* may also be.

[0131] The cytochrome P450 hydroxylase BorI shares greatest similarity to TyIHI, which catalyses the hydroxylation of an exocyclic methyl group of the tylosin macrolactone prior to addition of a deoxyhexose moiety (Fouces *et al.*, 1999). BorI is therefore believed to catalyse oxidation of the C12-methyl group during borrelidin biosynthesis. In agreement with this the *borI* mutant *S. parvulus* Tü4055/*borI::aac3(IV)* fails to produce borrelidin but accumulates a new product **14** (figure 6) that is less polar than borrelidin. **14** is readily transformed to borrelidin when fed to the *borE* mutant *S. parvulus* Tü4055/*borE::aac3(IV)* which lacks the ability to synthesise the PKS starter unit but maintains the rest of the borrelidin biosynthetic genes intact. Fermentation of *S. parvulus* Tü4055/*borI::aac3(IV)* followed by extraction and isolation provided ~30 mg of **14** (example 14). Full structural analysis of **14** identified it as 12-desnitrile-12-methylborrelidin (pre-borrelidin). This is consistent with the proposed role of BorI in borrelidin biosynthesis and provides a route to novel borrelidin analogues with a methyl group attached to C12 of the macrolactone ring.

[0132] The putative PLP dependent aminotransferase BorJ is believed to catalyse the introduction of a nitrogen atom into borrelidin at the activated C28-position, probably via a C12-formyl moiety. In agreement with this the *borJ* mutant *S. parvulus* Tü4055/*borJ::aac3(IV)* does not produce borrelidin and accumulates a new compound that is more polar than borrelidin. This new compound is not transformed to borrelidin when fed to mutant *S. parvulus* Tü4055/*borE::aac3(IV)* which indicates that it is probably a shunt metabolite rather than an intermediate in borrelidin biosynthesis. Fermentation of *S. parvulus* Tü4055/*borJ::aac3(IV)* allowed the isolation of 17 mg of the accumulated compound (example 15). Detailed structural analysis identified the accumulant as 12-desnitrile-12-carboxyl borrelidin **2**.

[0133] In addition to the compounds isolated from mutation of the borrelidin biosynthetic genes, 12-desnitrile-12-formyl borrelidin **15** is isolated from the fermentation supernatant of *S. parvulus* Tü113. The fermentation media and conditions used for these experiments differ from those we have described so far herein, but are designed to maximise the production of borrelidin. We propose that this altered medium, in combination with a drop in the dissolved oxygen concentration that is observed to occur during this specific fermentation, promoted the accumulation of **15**. **15** is readily transformed to borrelidin when fed to the mutant *S. parvulus* Tü4055/*borE::aac3(IV)* which lacks the ability to synthesise the PKS starter unit but maintains the rest of the borrelidin biosynthetic genes intact.

[0134] The above data lead us to propose a biosynthetic route to the nitrile moiety of borrelidin as presented in figure 6. The C12-methyl carbon of pre-borrelidin **14** is first oxidised by BorI to introduce an allylic hydroxyl group at C28 (**16**). This hydroxyl group is then converted to the formyl moiety attached to C12 (**15**) using a method selected from the group comprising: spontaneous oxidation (including oxidation mediated by some background enzyme) the action of a specific gene of the borrelidin biosynthetic gene cluster; candidate gene products are thus BorI itself, acting in a multifunctional manner and operating via the formation of a gem-diol structure at C12 followed by dehydration; or alternatively, via one of the oxidoreductase encoding genes such as *borC* or *borK*. The next step is anticipated to be BorJ-catalysed transamination of **15** in order to introduce a nitrogen atom at C28, in the form of an amine, through a pyridoxamine phosphate mediated process. The putative product amine **17** then undergoes oxidation, possibly spontaneously, but most probably by an enzymic activity such as BorI (certain parallels can be drawn to the biosynthesis of nitriles in plants (Celenza, 2001; Hahn *et al.*, 1999; Nielson and Møller, 1999)) or by the products of one of the oxidoreductase encoding genes,

e.g. *borC* or *bork*, or by a general oxidoreductase within the proteome.

[0135] In order to examine this proposed pathway in more detail a number of biotransformation experiments were performed using pre-borrelidin **14** as substrate for investigating the action of *borI*-K individually and in combination, using pEM4 as vector and *S. albus* J1074 (Chater & Wilde, 1980) as an expression strain. Expression of *borI* or *borJ* individually did not give borrelidin production on addition of **14**. The added **14** was only consumed during biotransformation with *borI* (and not in any of the control experiments); the **14** added was identified as being converted to the shunt metabolite **2**. However, co-expression of *borI* & *borJ* did convert the added **14** to borrelidin. It thus appears that either BorI or general proteome activities in *S. albus* are capable of oxidising the proposed amine intermediate **17** in the borrelidin biosynthetic pathway. In addition to the feeding of pre-borrelidin **14**, 12-desnitrile-12-carboxyl borrelidin **2** was also fed to the three strains described above. No conversion of **2** to borrelidin was observed in any of these experiments, reinforcing the idea that **2** is a shunt metabolite.

[0136] Detailed investigation of genomic DNA from three borrelidin producing strains, *S. rochei* ATCC23956, *S. parvulus* Tü113 and *S. parvulus* Tü4055, using numerous restriction digests and subsequent Southern Blot analysis, indicates that the borrelidin biosynthetic gene clusters of these three organisms are very closely conserved. It therefore appears that the borrelidin biosynthetic pathways of these strains are very similar. This assumption allows us to consider the data above, which are obtained from different strains, as applicable to a single biosynthetic pathway.

[0137] It is clear to one skilled in the art that manipulation of the genes involved in formation of the C12-nitrile moiety of borrelidin, for example *borI*, or *borJ*, is a generally useful method for the production of novel borrelidin related molecules and borrelidin derivatives with altered functionality at C12. In addition, the transfer of these genes to other organisms producing other natural or engineered polyketide products may allow the incorporation of nitrile moieties into such compounds.

[0138] In an extension of this work, disruptions in *borI* and *borJ* are separately made in the strain *S. parvulus* Tü4055/*borG::aac3(IV)* to give the doubly mutated strains *S. parvulus* Tü4055/*borG::aac3(IV)*/*borI::hyg* and *S. parvulus* Tü4055/*borG::aac3(IV)*/*borJ::hyg* (examples 12 & 13 respectively). These strains are fed alternative carboxylic acids, *trans*-cyclobutane-1,2-dicarboxylic acid, 2,3-dimethylsuccinic acid and 2-methylsuccinic acid, (as described above) and are found to produce the mutasynthetic borrelidin analogues carrying, either, a methyl (**21**, **22** and **23** respectively) or a carboxyl function at C12 (**24**, **25** and **26** respectively) in place of the nitrile group, and which are also derived from alternative starter units corresponding to the exogenously supplied carboxylic acids. This orthogonal library of new compounds is described in figure 11 and the observed UV chromophores and mass spectral data for each compound is shown.

EXAMPLES

General methods

[0139] Restriction enzymes, other molecular biology reagents, antibiotics and chemicals were purchased from standard commercial sources. Restriction endonuclease digestion and ligation followed standard methods (Sambrook, J. *et al.*, 1989).

Example 1: Fermentation of *S. parvulus* strains

[0140] The following method is generally useful for culturing *S. parvulus* for the production of borrelidin and/or borrelidin analogues:

[0141] A seed flask containing NYG medium (30 ml in a 250 ml Erlenmeyer flask) was inoculated from a working stock (0.5 ml). NYG medium contains, in deionised water: beef extract (0.3 %), Bacto peptone (0.5 %), glucose (1 %) and yeast extract (0.5 %). After 2 days shaking in a rotary incubator (2-inch throw; 30°C; 250 rpm) the resulting cream culture was used to inoculate PYDG production medium (30 ml in a 250 ml Erlenmeyer flask; 10 % inoculum). PYDG medium contains per litre of deionised water: peptonised milk nutrient (1.5 %), yeast autolysate (0.15 %), dextrin (4.5 %) and glucose (0.5 %) adjusted to pH 7.0. After 5 days shaking on a rotary incubator (2-inch throw; 30°C; 250 rpm) the culture was harvested for analysis as described in example 4, or for isolation purposes as required. For quantitative analysis these experiments were performed in triplicate.

[0142] The following method is useful for the feeding of exogenous carboxylic acids to *S. parvulus* strains:

[0143] The *S. parvulus* strain was grown as described above. After 24 hours growth in PYDG production medium, the carboxylic acid of choice was added as a 50 µl single aliquot (0.6 M solution in 70 % methanol after neutralization with 5 N NaOH). The resulting culture was harvested after 5 days total fermentation and analysed as described in example 4. For quantitative studies these experiments were performed in triplicate, and the equivalent fed and unfed WT strains served as controls.

Example 2: Cryopreservation of *S. parvulus* strains*Working stocks*

- 5 [0144] Working stocks of vegetative mycelia were prepared by mixing a 2 day old seed culture grown in NGY medium (0.5 ml) with cryopreservative (0.5 ml). Cryopreservative consists of 20 % glycerol and 10 % lactose in deionised water.

Spore stocks

- 10 [0145] Strains of *S. parvulus* were incubated on HA agar plates at 30°C. After 14 days the resulting spores from a single plate were harvested and suspended in of cryopreservative (1 ml). HA agar contains in deionised water: 0.4% yeast extract, 1% malt extract, 0.4 % dextrose and 1.5 % agar adjusted to pH 7.3.

Example 3: Cloning of the borrelidin biosynthetic gene cluster and disruption of *borA2* & *borA3*

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Cosmid library generation

- [0146] A cosmid library was constructed in pWE15 cosmid vector using the Gigapack® III Gold Packaging Extract kit according to the manufacturer's handbook (Stratagene). Chromosomal DNA was extracted from *S. parvulus* Tü4055 according to standard protocols (Kieser *et al.*, 2000) and treated with *Sau3AI* prior to cloning into pWE15. A number of the resulting *E. coli* transformants (3300) were picked and transferred to 96 well microtitre plates containing Luria Broth (LB) medium (0.1 ml per well) with ampicillin (100 µg/ml). The resulting clones were replica-plated to Luria agar (LA) plates containing ampicillin (100 µg/ml). After incubation overnight at 37°C colonies were transferred to nylon membrane filters for *in situ* colony hybridization analysis according to published protocols (Sambrook *et al.*, 1989).

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Library screening

- [0147] The cosmid library was screened using a probe that was generated using the DIG DNA Labelling and detection kit (Roche) according to the manufacturers instructions. The probe used was a *BglII*-*Bam*HI fragment (1.7 kbp) obtained from the gene that encodes module 6 of the third subunit of the oleandomycin PKS from *Streptomyces antibioticus* (Swan *et al.*, 1994).

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Disruption of the borrelidin biosynthetic gene cluster

- 35 [0148] Cosmids that gave a positive response when screened as described above were digested with *Bam*HI and fragments of less than 3 kbp were subcloned into pOJ260 (Bierman *et al.*, 1992). These were then used to transform protoplasts of *S. parvulus* Tü4055 as described in example 5. The resulting transformants were then assessed for the ability to produce borrelidin. Two clones were borrelidin non-producers; both were obtained from cosBor32A2 and contain sequence typical of a modular PKS. The remaining cosmids were then screened using probes obtained from the two
- 40 *Bam*HI fragments, which led to the identification of the overlapping cosmid cosBor19B9 that contained the remainder of the borrelidin biosynthetic cluster.

Sequencing of cosBor32A2 and cosBor19B9

- 45 [0149] The cosmids cosBor32A2 and cosBor19B9 were transformed into *E. coli* DH10B and the resulting clones grown at 37°C in 2xTY media (30 ml) containing ampicillin. After 15 hours the cells were harvested and Qiagen Tip 100 kits were used to prepare cosmid DNA. Approximately 5 µg of the cosmid DNA was digested with *Sau3AI* (1 U). Samples were taken at 2, 4, 6, 8 & 10 minute intervals after the enzyme was added and quenched into an equal volume of ice cold 0.5M EDTA. The samples were mixed and then analysed by gel electrophoresis, and those fragments between
- 50 1.5-2.0 kbp recovered from the gel. The fragments were cloned into linearised and dephosphorylated pHSG397 (Take-shita *et al.*, 1987), and transformed into *E. coli* DH10B. The resulting clones that contained insert were grown in 2xTY medium (2 ml) containing chloramphenicol (30 µg/ml) and purified using Wizard kits (Promega).

- [0150] DNA sequencing was carried out using an Applied Biosystems 800 Molecular Biology CATALYST robot to perform the dideoxy terminator reactions, which were then loaded into an ABI Prism 3700 automated sequencer (Applied Biosystems). The raw sequence data was processed using the Staden software package. Assembly and contig editing was performed using GAP (Genome Assembly Program) version 4.2 (Bonfield *et al.*, 1995). The GCG package (Devereux *et al.*, 1984) version 10.0 was used for sequence analysis.

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Example 4: Chemical analysis of *S. parvulus* strains

[0151] The following method is useful for analysing fermentations (see example 1) for the production of natural borrelidins and of engineered borrelidin analogues:

[0152] In a 2 ml Eppendorf tube, an aliquot of 5 day old fermentation broth (1 ml) was adjusted to pH-3 by the addition of 90 % formic acid (ca. 20 μ l). Ethyl acetate (1 ml) was added to the sample and mixed vigorously for 10 min using a vortex tray. The mixture was separated by centrifugation in a microfuge and the upper phase removed to a clean 2 ml Eppendorf tube. The ethyl acetate was removed by evaporation using a Speed-Vac. Residues were dissolved into methanol (250 μ l) and clarified using a microfuge. Analysis was performed on an Agilent HP1100 HPLC system as described below:

Injection volume:	50 μ l
Column stationary phase:	150 x 4.6 mm column, base-deactivated reversed phase silica gel, 3 μ m particle size (Hypersil C ₁₈ -BDS).
Mobile phase A:	10 % acetonitrile:90 % water, containing 10 mM ammonium acetate and 0.1 % TFA.
Mobile phase B:	90 % acetonitrile:10 % water, containing 10 mM ammonium acetate and 0.1 % TFA.
Mobile phase gradient:	T=0 min, 25%B; T=15, 100%B; T=19, 100%B; T=19.5, 25%B; T=25, 25%B.
Flow rate:	1 ml/min.
Detection:	UV at 258 nm (DAD acquisition over 190-600 nm); MS detection by electrospray ionisation over <i>m/z</i> range 100-1000 amu, with +/-ve ion mode switching.

Example 5: Protoplast transformation protocol for *S. parvulus* Tü4055

[0153] A seed flask containing tryptone soy broth (TSB) medium (10 ml in a 100 ml Erlenmyer flask) was inoculated from a working stock (0.15 ml). After 3 days shaking on a rotary incubator (30°C, 250 rpm), 5 ml of the culture was used to inoculate R5 medium (Kieser *et al.*, 2000) (50 ml in a 250 ml Erlenmeyer flask) that was then shaken on a rotary incubator for 24 hours (30°C, 250 rpm). The PEG mediated transformation of protoplasts was then performed according to standard published protocols (Kieser *et al.*, 2000).

Example 6: Replacement of borAT4 with rapAT2 - production of C10-desmethyl borrelidin

[0154] The borrelidin PKS AT4 domain is replaced with the AT2 domain of the rapamycin polyketide synthase as follows:

[0155] CosBor32A2 is digested with *EcoRI* and the 5429 bp band isolated. This is used as a template for PCR using the oligos CM410 (5'-AAAATGCATTCGGCCTGAACGGCCCCGCTGTCA-3') (SEQ ID No.44) and CM411 (5'-AAATGGCCAGCGAACCAACACCACACCACCA-3') (SEQ ID No.45). CM410 introduces an *NsiI* restriction site for cloning purposes and CM411 introduces an *MscI* site for use in the introduction of a heterologous AT. The ~1.1 kbp product is cloned into pUC18 digested with *SmaI* and dephosphorylated. The insert can ligate in two orientations and the reverse orientation is screened for by restriction enzyme analysis and the insert sequenced. One correct plasmid is designated pCJM462. Methylation deficient DNA (specifically dcm⁻) of pCJM462 and pCJR26 (Rowe *et al.* 1998) is isolated by passaging the plasmids through *E. coli* ET12567. Each plasmid is then digested with *MscI* and *XbaI* and the ~7.8 kbp fragment from pCJR26, containing the rapamycin AT2 and sequences downstream in pCJR26, is ligated to the ~3.8 kbp backbone generated by digestion of pCJM462. Plasmid pCJM463 is identified by restriction analysis.

[0156] CosBor32A2 is digested with *EcoRI* and *EcoRV* and the 2871 bp band isolated. This is used as a template for PCR using the oligos CM412 (5'-AAAGTCCTAGCGGCGGCCGCGGGTCGACCT-3') (SEQ ID No.46) and CM413 (5'-TTTAGATCTCGCGACGTCGCACGCGCCGAACGTCA-3') (SEQ ID No.47). CM412 introduces an *AvrII* restriction site that joins, in frame, the downstream borrelidin homology to the heterologous AT, and CM413 introduces a *BglII* site for cloning purposes. The ~1.1 kbp product is cloned into pUC18 digested with *SmaI* and dephosphorylated. The insert can ligate in two orientations and the reverse orientation is screened for by restriction enzyme analysis and the insert sequenced. One correct plasmid is designated pCJM464.

[0157] Plasmids pCJM463 and pCJM464 are digested with *AvrII* and *XbaI* and the ~1.1 kbp fragment from pCJM464 is ligated into the ~4.7 kbp backbone of pCJM463 to give pCJM465, which is identified by restriction enzyme analysis. pCJM465 contains the hybrid rapamycin AT2 with flanking regions of borrelidin sequence which provide homology for integration and secondary recombination.

[0158] Plasmid pCJM465 is digested with *NsiI* and *BglII* and the ~3 kbp fragment is cloned into pSL1180 previously digested with *NsiI* and *BamHI* to give pCJM466. Plasmid pCJM466 is then digested with *NsiI* and the apramycin cassette is incorporated on a *PstI* fragment from pEFBA (Lozano *et al.* 2000) to give the replacement vector pCJM467. pCJM467

is introduced into *S. parvulus* Tü4055 by protoplast transformation as described in example 5. Colonies resistant to apramycin (25 µg/ml) are initially identified, and then passaged several times through MA media without antibiotic selection in order to promote the second recombination (Fernandez *et al.* 1998). Several apramycin-sensitive colonies are isolated and analysed by PCR and Southern blot. The new mutant is named *S. parvulus* Tü4055/467.

[0159] *S. parvulus* Tü4055/467 is analysed as described in example 1 and shown to produce a mixture compounds with the correct UV spectrum. One of the new major components that is more polar than borrelidin has the correct retention time for 10-desmethyl borrelidin **3**. LCMS analysis indicates an *m/z* ratio for a compound that is 14 mass units lower than borrelidin as expected, and with an appropriate mass fragmentation pattern. Borrelidin itself is also produced, but at levels lower than the WT organism.

Example 7: Mutation of the methylmalonyl-CoA selective motif of borAT4 to generate 10-desmethyl borrelidin

[0160] Site directed mutagenesis of acyl transferase domains may also be used to alter the specificity of an AT. In this example the specificity of borAT4 is directed from methyl-malonyl-CoA towards malonyl-CoA. An amino acid motif has been identified (Reeves *et al.*, 2001; WO 02/14482) which directs the specificity of an AT. The motif YASH, as observed in borAT4, is found in methylmalonyl-CoA specific ATs and in this example it is altered to HAFH which is found in malonyl-CoA specific ATs.

[0161] CosBor32A2 is digested with *NcoI* and the 5167 bp band isolated. This is used as a template for PCR using the primers CM414 (5'-AAACTGCAGAGTCAACATCGGTCACACGCAGGC-3') (SEQ ID No.48) and CM415 (5'-AAAATGCATGATCCACATCGATACGACGCGCCCGA-3') (SEQ ID No.49). CM414 introduces a *PstI* restriction site for cloning purposes, and CM415 is a mutagenic primer covering the motif encoding region of the AT which will effect the amino acid changes and contains an *NsiI* site for cloning purposes. The ~1.1 kbp fragment is cloned into pUC18 digested with *SmaI* and dephosphorylated. The insert can ligate in either orientation and the forward orientation is screened for by restriction enzyme analysis and the insert sequenced. One correct plasmid is designated pCJM468.

[0162] A second PCR reaction is performed using the 5167 bp *NcoI* fragment of CosBor32A2 and the primers CM416 (5'-TAAATGCATTCCATTTCGGTGCAGGTGGAGTTGATCC-3') (SEQ ID No.50) and CM417 (5'-ATAGGATCCCCTC-CGGGTGCTCCAGACCGGCCACCC-3') (SEQ ID No.51). CM416 introduces an *NsiI* restriction site and is also a mutagenic primer covering the motif encoding region of the AT, and CM417 introduces a *BamHI* site for cloning purposes. The ~1.1 kbp fragment is cloned into pUC18 previously digested with *SmaI* and dephosphorylated. The insert can ligate in two orientations and the forward orientation is screened for by restriction enzyme analysis and the insert sequenced. One correct plasmid is designated pCJM469.

[0163] Plasmids pCJM468 and pCJM469 are digested with *NsiI* and *XbaI* and the ~1.1 kbp fragment from pCJM468 is ligated into the ~3.8 kbp backbone of pCJM469 to give pCJM470, which is identified by restriction enzyme analysis. pCJM470 contains the mutated motif of borAT4 with ~1.1 kbp of homologous DNA on either side which provide homology for integration and secondary recombination.

[0164] Plasmid pCJM470 is digested with *PstI* and *BamHI* and the ~2.2 kbp fragment is cloned into pSL1180 (Amersham Biosciences) previously digested with *PstI* and *BamHI* to give pCJM471. Plasmid pCJM471 is then digested with *PstI* and the apramycin cassette is incorporated on a *PstI* fragment from pEFBA (Lozano *et al.*, 2000) to provide the replacement vector pCJM472.

[0165] The replacement vector pCJM472 is introduced into *S. parvulus* Tü4055 by protoplast transformation as described in example 5. Colonies resistant to apramycin are initially identified, and then passaged several times through MA media without antibiotic selection in order to promote the second recombination (Fernandez *et al.*, 1998). Several apramycin-sensitive colonies are isolated and analysed by PCR and Southern blot, and one is selected that contains the new AT4 sequence containing the mutated motif and the *NsiI* site. The new mutant is named *S. parvulus* Tü4055/472.

[0166] *S. parvulus* Tü4055/472 is grown and analysed as described in example 1 and shown to produce a mixture of compounds with the correct UV profile for borrelidin. One of the new major components, that is more polar than borrelidin, has the correct retention time for authentic **3**. LCMS analysis indicates an *m/z* ratio for a compound that is 14 mass units lower than borrelidin as expected, and with an appropriate mass fragmentation pattern. Borrelidin itself is also produced, but at levels lower than the WT organism.

Example 8: Disruption of *borE* (*S. parvulus* Tü4055/*borE::aac3(IV)*)

[0167] In order to disrupt *borE*, an internal 761 bp fragment of the gene was amplified by PCR using primers B25A (5'-TTCTGCAGCCGCGCCTTCG-3') (SEQ ID No.81) and B25B (5'-AGAATTCGCCGCGCCGCTG-3') (SEQ ID No. 82) using cosBor32A2 as template. The product was purified, digested *PstI*-*EcoRI* and cloned into pOJ260ermE* which had been digested similarly, to provide pOJEd1. This approach was used in order to avoid possible polar effects. The vector pOJEd1 was introduced into *S. parvulus* Tü4055 by protoplast transformation as described in example 5, and colonies were selected for apramycin resistance on R5 and then on MA agar. The disruption was verified by Southern

hybridisation and the new mutant was named *S. parvulus* Tü4055/*borE::aac3(IV)*. Strain *S. parvulus* Tü4055/*borE::aac3(IV)* was grown, extracted and analysed as described in example 1. No borrelidin production was observed whereas a wild type control produced borrelidin as expected.

[0168] To verify that no polar effects were introduced a full-length copy of *borE* under the control of the *ermE** promoter was introduced *in trans* to the disrupted mutant. Full-length *borE* was amplified by PCR using the primers B7T1 (5'-GGCTGCAGACGCGGCTGAAG-3') (SEQ ID No.83) and B7T2 (5'-CCGGATCCCAGAGCCACGTC-3') (SEQ ID No.84) using cosBor32A2 as template. The 1216 bp product was purified, digested with *Pst*I-*Bam*HI and cloned into *Pst*I-*Xba*I digested pIJ2925 (Janssen & Bibb, 1993), along with a *Bam*HI-*Spe*I digested fragment from pLHyg containing the hygromycin resistance cassette, to generate pIJEH. A 2.8 kbp *Bam*HI fragment was excised from pIJEH and cloned into pEM4 (Quiros et al., 1998), which had been digested similarly, to give pborEH (in which the *borE* gene was cloned in the correct orientation for gene expression). pborEH and the control plasmid pEM4 were introduced into *S. parvulus* Tü4055/*borE::aac3(IV)* by protoplast transformation as described in example 5. The resulting strain *S. parvulus* Tü4055/*borE::aac3(IV)*/pborEH was analysed as described in example 1 and shown to produce borrelidin at a titre similar to a WT control; the control strain *S. parvulus* Tü4055/*borE::aac3(IV)*/pEM4 did not produce borrelidin.

[0169] Chemical complementation of *S. parvulus* Tü4055/*borE::aac3(IV)* with *trans*-1,2-dicyclopentane dicarboxylic acid, following the protocol described in example 1, demonstrated that the strain thus grown was capable of borrelidin production at $122 \pm 23\%$ of the WT parent control. Thus, *borE* is required for biosynthesis of *trans*-cyclopentane-1,2-dicarboxylic add.

Example 9: Disruption of *borG* (*S. parvulus* Tü4055/*borG::aac3(IV)*)

[0170] In order to disrupt *borG*, an internal region of 885 bp was amplified by PCR using the primers B23A (5'-ATCTGCAGCGGCATCGGTGT-3') (SEQ ID No.105) and B23B (5'-AGAATTCTCCACTGCGGTGCG-3') (SEQ ID No.106) and cosmid Bor32A2 as template. The resulting product was purified and the digested at the flanking sites *Pst*I-*Eco*RI and then subcloned into pOJ260P, downstream of the promoter *ermE**, to generate pOJGd1.

[0171] The vector pOJGd1 was introduced into *S. parvulus* Tü4055 by protoplast transformation as described in example 5. Colonies resistant to apramycin were selected on MA agar. The disruption was verified by Southern hybridisation and the new mutant was named *S. parvulus* Tü4055/*borG::aac3(IV)*. Strain *S. parvulus* Tü4055/*borG::aac3(IV)* was grown, extracted and analysed as described in example 1. Borrelidin production was compared to a wild type control. In addition, *S. parvulus* Tü4055/*borG::aac3(IV)* was chemically complemented with *trans*-1,2-dicyclopentane dicarboxylic acid, following the protocol described in example 1.

Example 10: Disruption of *borI* (*S. parvulus* Tü4055/*borI::aac3(IV)*)

[0172] The gene *borI* and surrounding DNA was amplified from cosBor19B9 using the PCR primers BP4501 (5'-CGTATGCATGGCGCCATGGA-3') (SEQ ID No.85) and BP4502 (5'-AGCCAATTGGTGCCTCCAG-3') (SEQ ID No.86). The 2.32 kbp product was purified, digested with *Nsi*I-*Mfe*I and cloned into pSL1180 digested *Nsi*I-*Eco*RI, to give plasmid pSLI. The apramycin resistance cassette was excised from pEFBA as an *Eco*RI fragment and cloned into pSLI digested with *Eco*RI, to give the plasmid pSLIA. Finally, the hygromycin resistance cassette was excised *Spe*I-*Pst*I from pLHyg and cloned into pSLIA which had been digested with *Nsi*I-*Spe*I to give plasmid pSLIr1.

[0173] The replacement vector pSLIr1 was introduced into *S. parvulus* Tü4055 by protoplast transformation as described in example 5. Colonies resistant to apramycin (25 µg/ml) were selected, and then passaged several times through MA media without selection. The replacement was verified by Southern hybridisation and the new mutant was named *S. parvulus* Tü4055/*borI::aac3(IV)*.

[0174] *S. parvulus* Tü4055/*borI::aac3(IV)* was grown and analysed as described in example 1. No borrelidin production was observed whereas several new compounds were observed at significantly lower levels. One of the less polar compounds displayed a UV absorbance maximum of 240 nm, and LCMS analysis indicated an *m/z* ratio 11 mass units lower than that for borrelidin, which is consistent with the presence of a methyl- rather than a nitrile-group at C12.

[0175] To verify that no polar effects were introduced a full-length copy of *borI* under the control of the *ermE** promoter was introduced *in trans* to the disrupted mutant. A 2.1 kb *Nsi*I-*Avr*II fragment containing *borI* was recovered from pSLI and subcloned into the *Pst*I-*Xba*I sites of pEM4, together with the *Nhe*I-*Spe*I fragment from pLHyg containing the hyg gene. Both fragments were subcloned in the same orientation generating pborIH. Plasmid pborIH and the control plasmid pEM4 were introduced into *S. parvulus* Tü4055/*borI::aac3(IV)* by protoplast transformation as described in example 5. The resulting strain *S. parvulus* Tü4055/*borI::aac3(IV)*/pborIH was analysed as described in examples 1 & 4, and shown to produce borrelidin at a titre similar to a WT control.

Example 11: Disruption of *borJ* (*S. parvulus* Tü4055/*borJ::aac3(IV)*)

[0176] The gene *borJ* and surrounding DNA was amplified from cosBor19B9 using the PCR primers BNHT1 (5'-GTCATGCATCAGCGCACCCG-3') (SEQ ID No.87) and BNHT2 (5'-GTGCAATTGCCCTGGTAGTC-3') (SEQ ID No.88). The 2.75 kbp product was purified, digested with *NsiI-MfeI* and cloned into pSL1180 that had been digested with *NsiI-EcoRI*, to give plasmid pSL. The hygromycin resistance cassette was excised from pLHyg as a *PstI-SpeI* fragment and cloned into pSL digested with *NsiI-SpeI*, to give pSLJH. Finally, the apramycin resistance cassette was excised from pEFBA with *SpeI-BamHI* and cloned into pSLJH that had been pre-digested with *AvrII-BglII* in order to remove a 453 bp fragment from *borJ*, to give plasmid pSLJr1.

[0177] The replacement vector pSLJr1 was introduced into *S. parvulus* Tü4055 by protoplast transformation as described in example 5. Colonies resistant to apramycin (25 g/ml) were selected, and then passaged several times through MA media without selection. The replacement was verified by Southern hybridisation. The new mutant was named *S. parvulus* Tü4055/*borJ::aac3(IV)*.

[0178] *S. parvulus* Tü4055/*borJ::aac3(IV)* was grown and analysed as described in example 1. No borrelidin production was observed whereas a new compound more polar than borrelidin was observed with a UV maximum at 262 nm. LCMS analysis indicated a parent compound of 508 amu, which is consistent with a carboxylic acid rather than a nitrile function at C12.

[0179] To verify that no polar effects were introduced a full-length copy of *borJ* under the control of the *ermE** promoter was introduced *in trans* to the disrupted mutant. A 2.4 kb *NsiI-SphI* fragment from pSLJ containing *borJ* was subcloned into the *PstI-XbaI* sites of pEM4, together with the *hyg* gene as a *SphI-SpeI* fragment from pLHyg; both fragments were subcloned in the same orientation as the transcription of the genes. The final construct was designed pborJH. Plasmid pborJH and the control plasmid pEM4 were introduced into *S. parvulus* Tü4055/*borJ::aac3(IV)* by protoplast transformation as described in example 5. The resulting strain *S. parvulus* Tü4055/*borJ::aac3(IV)*/pborJH was analysed as described in examples 1 & 4, and shown to produce borrelidin at a titre similar to a WT control.

Example 12: Disruption of *borG* and *borI* (*S. parvulus* Tü4055/*borG::aac3(IV)*/*borI::hyg*)

[0180] The *hyg* gene is isolated from pLHyg as an *EcoRV* fragment and cloned into pSLI (example 10) digested with *EcoRI* and treated with Klenow fragment to give pSLIH; the *hyg* gene is cloned in the same orientation as *borI*. pSLIH is introduced into *S. parvulus* Tü4055/*borG::aac3(IV)* by protoplast transformation, as described in example 5, and selected for both apramycin and hygromycin resistance, and is then passaged several times through MA media without selection in order to promote double recombination. Apramycin and hygromycin resistant colonies are analysed by Southern hybridisation and PCR to verify the replacement.

Example 13: Disruption of *borG* and *borJ* (*S. parvulus* Tü4055/*borG::aac3(IV)*/*borJ::hyg*)

[0181] The *hyg* gene is isolated from pLHyg as an *EcoRV* fragment and cloned into pSLJ (example 11) digested with *AvrII-BglIII* and treated with Klenow, to give pSLJH; the *hyg* gene is cloned in the same orientation as *borI*. pSLJH is introduced into *S. parvulus* Tü4055/*borG::aac3(IV)* by protoplast transformation, as described in example 5, and selected for both apramycin and hygromycin resistance, and is then passaged several times through MA media without selection in order to promote double recombination. Apramycin and hygromycin resistant colonies are analysed by Southern hybridisation and PCR to verify the replacement.

Example 14: Production of 12-desnitrile-12-methyl borrelidin 14 (pre-borrelidin)

[0182] Working stocks of *S. parvulus* Tü4055/*borI::aac3(IV)* (0.5 ml) were inoculated into primary vegetative pre-cultures of NYG as described in example 1. Secondary pre-cultures were prepared (as example 1 but with 250 ml NYG in 2 l Erlenmeyer flasks). PYDG production medium (4 l), prepared as in example 1 and with 0.01 % Plutronic L0101 added to control foaming, was inoculated with secondary pre-culture (12.5 % inoculum). A second fermenter containing centre-point medium (4 l) and 0.01 % Plutronic L0101 to control foaming, was set up in parallel and was also inoculated with secondary pre-culture (12.5 % inoculum). Centre-point production medium contains per litre of deionised water: Tesco's skimmed milk powder (1.5 %), Avidex W-80 (4.5 %), glucose (0.5 %) and yeast autolysate (0.15 %) adjusted to pH 7.0 with 5 M NaOH.

[0183] These batches were each allowed to ferment in a 7 l Applikon fermenter for 6.5 days at 30 °C. Airflow was set at 0.75 vvm (volume per volume per minute), with tilted baffles and the impeller speed controlled between 400 and 800 rpm to maintain dissolved oxygen tension at or above 30 % of air saturation. No further antifoam was added. At 22 hours into the fermentation the starter acid, *trans*-cyclopentane-1,2-dicarboxylic acid, was added as a neutralised solution of 1:1 MeOH / 5 M NaOH, through an in-line filter (0.22 µm). The final concentration in the fermenter vessel of exogenous

starter acid was 0.5 mM.

[0184] After 6.5 days of fermentation the broths were combined and acidified to pH 3.5 with concentrated HCl (~ 6 ml), then clarified by centrifugation at 3,500 rpm for 10 minutes. The supernatant was extracted into ethyl acetate (3 x 1 volume equivalent for 4 hours each) and the cell pellet left to steep in methanol (2 x 1.5 litres for 4 hours each). The organics were combined and removed under reduced pressure to yield a tarry gum. The gum was re-suspended in 0.1 M Borax buffer (500 ml at pH 9.4) and washed with hexanes (500 ml) and ethyl acetate (500 ml). The aqueous layer was then acidified with concentrated HCl to pH 3.5 and extracted with ethyl acetate (3 x 500 ml), which were combined and taken to dryness. The resultant gum was dissolved in methanol (15 ml), diluted with water (285 ml) and loaded under gravity onto a C₁₈-reversed-phase cartridge (50 g, prepared in 5 % aqueous methanol). The cartridge was washed with 20 % and 50 % aqueous methanol (300 ml each) and eluted with 100 % methanol (500 ml). This last fraction was taken to dryness under reduced pressure to yield a black gummy-oil (600 mg) that was taken up in methanol. This residue was finally purified by sequential preparative reversed-phase HPLC (eluted with the mobile phases used in example 4, without added TFA, running isocratically at 40 %B). Active fractions were combined and desalted on a C₁₈-cartridge (1 g), to yield 28 mg of a dark oil (3.5 mg/l isolated yield). Table 12 summarises the ¹H and ¹³C NMR chemical shift data for 12-desnitrile-12-methyl borrelidin **14** in CDCl₃.

Table 12

Position	δ_{H} (ppm)	Multiplicity	Coupling (Hz)	δ_{C} (ppm)
1	-	-	-	174.5
2a	2.29	m	-	37.8
2b	2.26	m	-	-
3	3.85	dt	9.0, 3.0	71.9
4	1.83	m	-	35.1
5a	1.19	bt	13.5	43.6
5b	0.91	m	-	-
6	1.75	m	-	27.0
7a	1.08	m	-	49.2
7b	0.88	m	-	-
8	1.69	m	-	26.5
9a	0.97	m	-	38.3
9b	0.45	t	12.5	-
10	1.62	m	-	34.1
11	3.53	d	9.0	85.7
12	-	-	-	138.4
13	5.84	d	11.0	127.7
14	6.28	ddd	14.5, 11.0, 1.0	129.6
15	5.48	ddd	14.5, 10.5, 3.5	129.9
16a	2.53	m	-	39.1
16b	2.22	m	-	-
17	5.07	ddd	11.0, 8.0, 3.0	76.5
18	2.52	m	-	48.0
19a	1.92	m	-	30.4
19b	1.32	m	-	-
20a	1.74	m	-	26.2
20b	1.71	m	-	-

(continued)

Position	δ_{H} (ppm)	Multiplicity	Coupling (Hz)	δ_{C} (ppm)
21a	1.96	m	-	32.0
21b	1.84	m	-	-
22	2.45	m	8.0	49.3
23	-	-	-	182.3
4-CH ₃	0.78	d	6.5	18.5
6-CH ₃	0.77	d	6.5	18.8
8-CH ₃	0.75	d	6.5	20.6
10-CH ₃	0.94	d	6.5	16.3
12-CH ₃	1.64	s	-	11.4
Chemical shifts are referenced to CDCl ₃ (for ¹ H at 7.26 ppm and for ¹³ C at 77.0 ppm)				

Example 15: Production of 12-desnitrile-12-carboxy borrelidin **2**

[0185] Working stocks of *S. parvulus* Tü4055/*borJ::aac3(IV)* (0.5 ml) were inoculated into primary vegetative pre-cultures of NYG as described in example 1. Secondary pre-cultures were prepared (as example 1 but with 250 ml NYG in 2 l Erlenmeyer flasks). PYDG production media (4 L), prepared as in example 1 and with 0.01 % Plutronic L0101 added to control foaming, was inoculated with the entire secondary pre-culture (10 % inoculum). This was allowed to ferment in a 7 L Applikon fermenter for 6 days at 30 °C. Airflow was set at 0.75 vvm, with tilted baffles and the impeller speed controlled between 250 and 600 rpm to maintain dissolved oxygen tension at or above 30 % of air saturation. No further antifoam was added. A second fermentation was performed exactly as above, but which was batch fed with 0.2 mol of glucose as an aqueous solution every 12 hours from 60 hours post-inoculation.

[0186] After 6 days the fermentations were harvested and combined. The broth was clarified by centrifugation (3,500 rpm, 10 minutes) and the resultant supernatant acidified with 10 M HCl (aq) to pH ~ 3.5. This solution was then extracted into ethyl acetate by stirring (3 x 1 volume equivalent for 4 hours each). The cell pellet was extracted twice by steeping the cells in 1:1 methanol / ethyl acetate (500 ml). All the organics were combined and removed under reduced pressure to yield an aqueous slurry. The slurry was diluted to 500 ml with water, acidified to pH ~ 3.5 with 10 M HCl and extracted with 0.1 M borax (3 x 300 ml). The organics were concentrated under reduced pressure to ~ 300 ml and extracted with 0.1 M borax (3 x 150 ml, pH = 9.4). The combined borax solutions were acidified with 10 M HCl to pH ~ 3.5 and extracted with 6 x 300 ml of ethyl acetate. Analytical HPLC demonstrated that some of the accumulant still resided in the borax solution and so this was loaded, under gravity, onto a C₁₈-reverse-phase cartridge (50 g). The cartridge was washed with water and the accumulant eluted in 100 % methanol. The organics containing the accumulant were combined and reduced to a 40 ml methanolic solution. This was loaded onto a Sephadex LH-20 column (70 g, swelled overnight in methanol, column 60 cm x 2.5 cm), which was developed with 100 % methanol; the active fractions were combined and taken to dryness. The material was then further processed by preparative reversed-phase HPLC (eluted with the mobile phases used in example 4, without added TFA, running isocratically at 40 %B). The combined active fractions were taken to dryness, dissolved in methanol (4 ml) and diluted with water (200 ml). This mixture was split into 2 equal fractions and each loaded, under gravity, onto a C₁₈-reverse-phase cartridge (20 g). The columns were then eluted with 3 column volumes of 5 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 75 %, 90 % and 100 % aqueous methanol. The accumulant eluted in all fractions from 60 % to 100 % methanol, which were combined and taken to dryness. The accumulant (dissolved in DMSO) was then finally purified by sequential preparative reversed-phase HPLC (eluted with the mobile phases used in example 4, without added TFA, running isocratically at 40 %B). Active fractions were combined and desalted on a C₁₈-cartridge (1 g), to yield 17 mg of a brown oil (2.1 mg/l isolated yield). Table 13 summarises the ¹H and ¹³C NMR chemical shift data for 12-desnitrile-12-carboxy borrelidin **2** in *d*₄-methanol.

Table 13

Position	δ_{H} (ppm)	Multiplicity	Coupling (Hz)	δ_{C} (ppm)
1	-	-	-	173.27
2a	2.40	dd	15.8, 4.1	39.31

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(continued)

Position	δ_H (ppm)	Multiplicity	Coupling (Hz)	δ_C (ppm)
2b	2.29	dd	15.8, 8.2	
3	3.87	m		71.64
4	1.80	m		36.51
5a	1.29	m		44.24
5b	0.90	m		
6	1.59	m		27.48
7a	1.09	m		~49.0*
7b	1.03	m		
8	1.72	m		28.17
9a	1.12	m		38.42
9b	0.79	m		
10	2.03	m		36.43
11	3.90	m		81.95
12	-	-	-	132.35
13	6.43	d	11.0	140.83
14	6.96	dd	14.5, 11.5	130.91
15	5.91	ddd	15.0, 9.5, 5.0	138.93
16a	2.61	m	15.0	38.57
16b	2.36	m		
17	5.04	m		77.40
18	2.50	m		49.80
19a	1.90	m		30.59
19b	1.32	m		
20a	1.85	m		26.34
20b	1.41	m		
21a	1.97	m		32.40
21b	1.75	m		
22	2.52	m		~48.0*
23	-	-	-	180.27
4-CH ₃	0.83	d	7.0	18.76
6-CH ₃	0.80	d	6.0	17.06
8-CH ₃	0.81	d	6.5	20.60
10-CH ₃	0.93	d	6.5	16.61
12-CO ₂ H -		-	-	170.49
Chemical shifts are referenced to methanol (for ¹ H at 3.35 ppm (quintet) and for ¹³ C at 49.0 ppm (septet)); *Obscured by solvent signal, d ₄ -methanol.				

Example 16: Production by mutasynthesis of 17-des-(cyclopentane-2'-carboxylic acid)-17-(cyclobutane-2'-carboxylic acid)borrelidin **18**

[0187] Working stocks of *S. parvulus* Tü4055/*borE::aac3(IV)* (0.5 ml) were inoculated into primary vegetative pre-cultures of NYG as described in example 1. Secondary pre-cultures were prepared (as example 1 but with 250 ml NYG in 2 l Erlenmeyer flasks). PYDG production medium (4 l), prepared as in example 1 and with 0.01 % Plutronic L0101 added to control foaming, was inoculated with secondary pre-culture (12.5 % inoculum). Two further bioreactors were set up in the same manner. These batches were each allowed to ferment in a 7 l Applikon fermenter for 5 days at 30 °C. Airflow was set at 0.75 vvm (volume per volume per minute), with tilted baffles and the impeller speed controlled between 400 and 700 rpm to maintain dissolved oxygen tension at or above 30 % of air saturation. No further antifoam was added. At 22 hours into the fermentation the starter acid, *trans*-cyclobutane-1,2-dicarboxylic acid, was added as a neutralised solution of 1:1 MeOH / 5 M NaOH. The final concentration in the fermenter vessel of exogenous starter acid was 0.5 mM.

After 5 days of fermentation the broths were combined and acidified to pH 4.0 with concentrated HCl, then clarified by centrifugation at 3,500 rpm for 10 minutes. The supernatant was absorbed onto diaion HP-20SS resin (1 l), which had been pretreated with methanol (2 l) and then 5 % aqueous methanol (2 l), by filtration at a rate of approximately 100 ml/min. The resin was then eluted with 20 % aqueous methanol (2.5 l) and then 80 % aqueous acetone (4.5 l). The organic solvent was removed from the aqueous acetone and the resultant aqueous slurry (1 litre) extracted into ethyl acetate (3 x 1 l). The organics were combined and reduced *in vacuo* to yield a yellow/brown oil (1.7 g). Meanwhile, the cell pellet left to steep in methanol-ethyl acetate, 1:1 (3 x 1 l for 4 hours each), and the resultant organic supernatants reduced *in vacuo* to yield an aqueous slurry (400 ml). The particulate matter was dissolved in methanol (50 ml), and added back to the aqueous slurry, which was made up to 500 ml with water. This slurry was absorbed onto diaion HP-20SS resin (300 ml), that had been pretreated with methanol (500 ml) and then 5 % aqueous methanol (500 ml). The resin was then eluted with 20 % aqueous methanol (1 l) and then 80 % aqueous acetone (1.5 l). The organic solvent was removed from the aqueous acetone and the resultant aqueous slurry (made up to 750 ml) extracted into ethyl acetate (3 x 750 ml). The organics were combined and reduced *in vacuo* to yield a yellow/brown oil (1.7 g). The crude extracts were combined (3.4 g), dissolved in ethyl acetate (10 ml), then adsorbed onto a silica column (5 cm ID x 10 cm, treated with EtOAc), and eluted with EtOAc. The active fractions were combined and the solvent removed *in vacuo* to yield a brown gum (1.08 g). This residue was finally purified by sequential preparative reversed-phase HPLC (eluted with the mobile phases used in example 4, without added TFA, running from 25 % B to 75 % B over 25 minutes with a linear gradient). Active fractions were combined and desalted on a C₁₈-cartridge (5 g), to yield 83.9 mg (or 7.0 mg / l isolated yield). The ¹³C-NMR spectrum of **18** is shown in table 14

Table14:

δ _c (ppm)	Position	δ _c (ppm)	Position
177.1	COOH (C22)	37.3	9
172.2	1	35.7	4
144.0	13	35.1	10
138.7	15	34.4	16
126.9	14	30.9	18
118.3	12	27.3	6
115.8	CN	26.2	8
75.5	17	21.7	20
73.1	11	21.0	21
69.7	3	20.1	8-Me
47.6	5	18.1	6-Me
43.1	7	16.9	4-Me
40.1	19	14.9	10-Me

(continued)

δ_c (ppm)	Position	δ_c (ppm)	Position
40.0	2		
¹³ C-NMR assignment for 18 , in CDCl ₃ , using that carbon signal as reference at $\delta_c = 77.7$ ppm			

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	Val	Arg	Gly	Val	Ala	Ser	Ser	Ala	Ser	Gly	Gly	Leu	Ala	Phe	Val	Phe	
	545					550					555					560	
	Ala	Gly	Gln	Gly	Ser	Gln	Arg	Leu	Gly	Met	Gly	Arg	Gly	Leu	Tyr	Glu	
50					565					570					575		
	Arg	Phe	Pro	Val	Phe	Ala	Glu	Ala	Phe	Asp	Glu	Val	Cys	Gly	Arg	Val	
				580					585					590			
55	Gly	Pro	Gly	Val	Arg	Glu	Val	Val	Phe	Gly	Ser	Asp	Ala	Gly	Glu	Leu	
			595					600					605				

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	Asp	Arg	Thr	Val	Trp	Ala	Gln	Ala	Gly	Leu	Phe	Ala	Leu	Glu	Val	Ala	
	610					615						620					
5	Leu	Phe	Arg	Leu	Leu	Glu	Ser	Trp	Gly	Val	Arg	Pro	Gly	Cys	Leu	Ile	
	625					630					635					640	
	Gly	His	Ser	Val	Gly	Glu	Leu	Ser	Ala	Ala	Cys	Val	Ala	Gly	Leu	Trp	
					645					650					655		
10	Ser	Leu	Glu	Asp	Ala	Cys	Arg	Val	Val	Ala	Ala	Arg	Ala	Arg	Leu	Met	
				660					665					670			
	Gln	Ala	Leu	Pro	Ala	Gly	Gly	Val	Met	Val	Ala	Val	Arg	Ala	Glu	Ala	
				675				680					685				
15	Gly	Glu	Leu	Ala	Gly	Phe	Leu	Gly	Glu	Asp	Val	Val	Ile	Ala	Ser	Val	
	690					695					700						
	Asn	Ala	Pro	Gly	Gln	Val	Val	Ile	Ala	Gly	Pro	Glu	Gly	Gly	Val	Glu	
	705				710						715					720	
20	Arg	Val	Val	Ala	Ala	Cys	Gly	Ala	Arg	Ser	Arg	Arg	Leu	Ala	Val	Ser	
				725						730					735		
	His	Ala	Phe	His	Ser	Pro	Leu	Val	Glu	Pro	Met	Leu	Gly	Glu	Phe	Arg	
				740				745					750				
25	Arg	Val	Val	Glu	Ser	Val	Ala	Phe	Gly	Val	Pro	Ser	Leu	Arg	Val	Val	
				755				760					765				
	Ser	Asn	Val	Thr	Gly	Ala	Trp	Val	Asp	Pro	Glu	Glu	Trp	Gly	Thr	Pro	
	770				775					780							
30	Glu	Tyr	Trp	Val	Arg	Gln	Val	Arg	Glu	Pro	Val	Arg	Phe	Ala	Asp	Gly	
	785				790					795						800	
	Val	Ala	Thr	Leu	Leu	Asp	Ala	Gly	Val	Arg	Thr	Phe	Val	Glu	Leu	Gly	
				805				810					815				
35	Pro	Ala	Gly	Ala	Leu	Thr	Ser	Met	Val	Ser	His	Cys	Ala	Asp	Ala	Thr	
				820				825					830				
	Ala	Thr	Ser	Val	Thr	Ala	Val	Pro	Thr	Leu	Arg	Pro	Asp	His	Asp	Glu	
				835				840					845				
40	Ser	Arg	Thr	Val	Leu	Ser	Ala	Ala	Ala	Ser	Leu	Tyr	Val	Gln	Gly	His	
	850				855					860							
	Pro	Val	Asp	Trp	Ala	Pro	Leu	Phe	Pro	Arg	Ala	Arg	Thr	Val	Asp	Leu	
	865				870				875					880			
45	Pro	Thr	Tyr	Pro	Phe	Gln	His	Gln	His	Tyr	Trp	Leu	Asp	Val	Pro	Pro	
				885				890					895				
	Leu	Phe	Thr	Ala	Ser	Ser	Ala	Ala	Gln	Asp	Gly	Gly	Trp	Arg	Tyr	Arg	
				900				905					910				
50	Ile	His	Trp	Arg	Arg	Leu	Gly	Thr	Arg	Asp	Ser	Gly	Asp	Arg	Leu	Ser	
				915				920					925				
	Gly	Arg	Trp	Leu	Leu	Leu	Val	Pro	Glu	Ser	Asp	Gly	Thr	Glu	Pro	Trp	
	930				935			940									
55	Val	Glu	Gly	Ala	Glu	Lys	Met	Leu	Ala	Glu	Arg	Gly	Cys	Glu	Val	Val	

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	945		950		955		960
	His Val Pro Ile Ala Thr Ala Asp Arg Asp Ala Met Val Gly Ala						
		965			970		975
5	Val Arg Glu Ser Val Glu Asp Gly Arg Val Asp Gly Val Leu Ser Leu						
		980		985			990
	Leu Ala Leu Asp Gly Arg Pro His Pro Asp Ala Ala Ala Val Pro Thr						
		995	1000			1005	
10	Gly Leu Val Ala Thr Ala Gln Val Val Gln Val Ser Asp Glu Leu Gly						
		1010	1015			1020	
	Ile Gly Pro Leu Trp Val Ala Thr Arg Gln Ala Val Ser Val Asp Gly						
		1025	1030		1035		1040
15	Ala Asp Glu Ala Asp Gly Ala Gly Arg Thr Arg Lys Ala Asp Asp Pro						
		1045		1050			1055
	Ala Asp Val Ala Gln Ala Ala Val Trp Gly Leu Gly Arg Val Ala Ala						
		1060		1065			1070
20	Leu Glu Lys Pro Arg Leu Trp Gly Gly Leu Val Asp Leu Pro Ala Arg						
		1075		1080			1085
	Ala Asp Glu Arg Met Arg Asp Leu Val Ala Gln Ala Leu Thr Ala Pro						
		1090		1095			1100
25	Asp Ala Glu Asp Gln Leu Ala Val Arg Ala Asp Gly Ile Ala Val Arg						
		1105		1110		1115	1120
	Arg Leu Val Arg Ser Ala Ala Ser Ala Pro Ala Asp Asp Trp Gln Pro						
		1125			1130		1135
30	Ser Gly Thr Val Leu Val Thr Gly Gly Thr Gly Gly Val Gly Ala Asn						
		1140		1145			1150
	Val Ala Arg Trp Leu Val Thr Gln Asp Ile Gln His Leu Leu Leu Val						
		1155		1160			1165
35	Ser Arg Arg Gly Pro Asp Ala Pro Gly Ala Ala Glu Leu Leu Ala Glu						
		1170		1175			1180
	Leu Ser Ala Ser Gly Thr Ser Val Thr Ile Glu Pro Cys Asp Val Thr						
		1185		1190		1195	1200
40	Asp Ala Asp Ala Val Arg Arg Leu Ile Gly Ala Val Pro Ala Glu Arg						
		1205		1210			1215
	Pro Leu Ser Thr Val Val His Ala Ala Gly Val Leu Asp Asp Cys Leu						
		1220		1225			1230
45	Ile Asp Ala Leu Thr Pro Gln Arg Leu Ala Ala Ala Leu Glu Val Lys						
		1235		1240			1245
	Ala Lys Gly Ala Leu Asn Leu His Glu Ala Ala Gly Glu Ala His Leu						
		1250		1255			1260
50	Val Leu Phe Ser Ser Leu Ala Gly Thr Thr Gly Thr Lys Gly Gln Gly						
		1265		1270		1275	1280
	Asn Tyr Ala Ala Ala Asn Ala Tyr Leu Asp Ala Leu Ala Glu Arg Arg						
		1285		1290			1295
55							

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Arg Ala Asp Gly Leu Pro Ala Thr Ser Val Ala Trp Gly Ala Trp Gln
 1300 1305 1310
 5 Gly Ala Gly Met Val Ala Asp Ala Ala Val Ala His Arg Thr Arg Arg
 1315 1320 1325
 Tyr Gly Leu Pro Leu Met Ser Pro Asp Arg Ala Val Ala Thr Leu Arg
 10 1330 1335 1340
 Gln Val Met Ala Glu Pro Val Ala Thr Gln Val Val Ala Asp Val Asp
 1345 1350 1355 1360
 Trp Gln Arg Phe Val Ala Asp Phe Thr Ala Val Arg Pro Ser Arg Leu
 15 1365 1370 1375
 Leu Ala Asp Leu Pro Glu Val Arg Ser Leu Gly Glu Gln Arg Lys Asp
 1380 1385 1390
 20 Gly Pro Gly Gly Gln Gly Glu Glu Asp Gly Leu Ala Ser Lys Leu Ala
 1395 1400 1405
 Ala Leu Pro Glu Ala Asp Arg Arg Arg Ala Val Leu Asp Leu Val Glu
 1410 1415 1420
 25 Glu Leu Val Leu Gly Val Leu Gly His Glu Thr Arg Ala Ala Ile Gly
 1425 1430 1435 1440
 Pro Asp Ser Ser Phe His Ala Ile Gly Phe Asp Ser Leu Thr Ala Val
 30 1445 1450 1455
 Glu Leu Arg Asn Leu Leu Thr Val Arg Leu Gly Met Lys Leu Pro Ala
 1460 1465 1470
 Thr Leu Val Tyr Asp His Pro Thr Leu Ser Ser Leu Ala Asp His Leu
 35 1475 1480 1485
 His Glu Gln Leu Val Ile Asp Gly Thr Pro Met Thr Asp Thr Ala Ala
 1490 1495 1500
 40 Asp Leu Leu Ala Glu Leu Asp Ala Leu Ala Ala Arg Leu Ala Ala Val
 1505 1510 1515 1520
 Gly Leu Glu Pro Glu Ala Arg Ala Arg Ile Gly Arg Arg Leu Lys Asp
 1525 1530 1535
 45 Met Gln Thr Ala Cys Glu Pro Arg Ser Glu Ser Ser Arg Asp Leu Lys
 1540 1545 1550
 Ser Ala Ser Arg Thr Glu Val Leu Asp Phe Leu Thr Asn Glu Leu Gly
 50 1555 1560 1565
 Ile Ser Arg
 1570

55 <210> 4
 <211> 3500
 <212> PRT
 <213> Streptomyces parvulus Tü4055

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<400> 4

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5	1				5					10					15	

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	Asn	Leu	Gln	Glu	Ala	Arg	Gln	Arg	Val	His	Glu	Leu	Glu	Glu	Ser	Glu	
				20					25					30			
5	Arg	Glu	Pro	Ile	Ala	Ile	Val	Gly	Met	Ser	Cys	Arg	Leu	Pro	Gly	Gly	
			35					40					45				
	Val	Asn	Ser	Pro	Glu	Glu	Phe	Trp	Ser	Leu	Leu	Glu	Ala	Gly	Thr	Asp	
		50					55					60					
10	Ala	Val	Ser	Glu	Phe	Pro	Arg	Asp	Arg	Gly	Trp	Asp	Val	Glu	Arg	Leu	
	65					70					75					80	
	Tyr	Asp	Pro	Asp	Pro	Asp	Ala	Pro	Gly	Lys	Ser	Tyr	Val	Arg	Glu	Gly	
					85					90					95		
15	Gly	Phe	Leu	Asp	Gly	Ala	Gly	Arg	Phe	Asp	Pro	Ala	Phe	Phe	Gly	Ile	
				100				105						110			
	Ser	Pro	Arg	Glu	Ala	Val	Val	Met	Asp	Pro	Gln	Gln	Arg	Leu	Leu	Leu	
			115					120					125				
20	Glu	Cys	Ser	Trp	Glu	Ala	Ile	Glu	Arg	Ser	Arg	Ile	Asp	Pro	Lys	Thr	
		130					135					140					
	Leu	His	Gly	Ser	Arg	Ala	Gly	Val	Phe	Val	Gly	Ser	Asn	Gly	Gln	Asp	
	145					150					155					160	
25	Tyr	Gly	Thr	Leu	Leu	Leu	Arg	Ala	Asp	Asp	Arg	Ser	His	Ala	Tyr	Leu	
				165						170					175		
	Ala	Thr	Gly	Ala	Ser	Ala	Ser	Val	Leu	Ser	Gly	Arg	Ile	Ser	Tyr	Thr	
				180					185					190			
30	Leu	Gly	Leu	Glu	Gly	Pro	Ala	Val	Thr	Ile	Ser	Thr	Ala	Cys	Ser	Ser	
			195					200					205				
	Ser	Leu	Val	Ala	Leu	His	Leu	Ala	Ala	Arg	Ala	Leu	Arg	Ala	Gly	Glu	
		210					215					220					
35	Cys	Glu	Leu	Ala	Leu	Ala	Gly	Gly	Val	Thr	Val	Met	Pro	Thr	Thr	Arg	
	225					230					235					240	
	Leu	Phe	Glu	Val	Phe	Ser	Arg	Gln	Arg	Gly	Leu	Ala	Gly	Asp	Gly	Arg	
				245						250					255		
40	Cys	Lys	Ala	Phe	Ala	Ala	Gly	Ala	Asp	Gly	Thr	Gly	Trp	Gly	Glu	Gly	
			260						265					270			
	Val	Gly	Val	Leu	Val	Leu	Glu	Arg	Leu	Ser	Val	Ala	Arg	Arg	Asn	Gly	
			275					280					285				
45	His	Arg	Val	Leu	Ala	Val	Val	Arg	Gly	Ser	Ala	Val	Asn	Gln	Asp	Gly	
		290					295					300					
	Ala	Ser	Asn	Gly	Leu	Thr	Ala	Pro	Asn	Gly	Pro	Ser	Gln	Gln	Arg	Val	
	305					310					315					320	
50	Ile	Arg	Ala	Ala	Leu	Ala	Ser	Ala	Arg	Leu	Ala	Pro	Glu	Asp	Val	Asp	
				325						330					335		
	Ala	Val	Glu	Ala	His	Gly	Thr	Gly	Thr	Ser	Leu	Gly	Asp	Pro	Ile	Glu	
			340					345					350				
55	Ala	Gln	Ala	Leu	Leu	Ala	Thr	Tyr	Gly	Arg	Gly	Arg	Asp	Ala	Glu	Arg	

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	Ser Val Asn Ala Pro Gly Gln Val Val Ile Ala Gly Pro Glu Gly Gly	705	710	715	720
5	Val Glu Arg Val Val Ala Ala Cys Gly Ala Arg Ser Arg Arg Leu Ala	725	730	735	
	Val Ser His Ala Phe His Ser Pro Leu Val Glu Pro Met Leu Gly Glu	740	745	750	
10	Phe Arg Arg Val Val Glu Ser Val Ala Phe Gly Val Pro Ser Leu Arg	755	760	765	
	Val Val Ser Asn Val Thr Gly Ala Trp Val Asp Pro Glu Glu Trp Gly	770	775	780	
15	Thr Pro Glu Tyr Trp Val Arg Gln Val Arg Glu Pro Val Arg Phe Ala	785	790	795	800
	Asp Gly Val Ala Thr Leu Leu Asp Ala Gly Val Arg Thr Phe Val Glu	805	810	815	
20	Leu Gly Pro Ala Gly Thr Leu Thr Ser Met Val Ser His Cys Ala Asp	820	825	830	
	Ala Thr Ala Thr Ser Val Thr Ala Val Pro Thr Leu Arg Pro Asp His	835	840	845	
25	Asp Glu Ser Arg Thr Val Leu Ser Ala Ala Ala Ser Leu Tyr Val Gln	850	855	860	
	Gly His Pro Val Asp Trp Ala Pro Leu Phe Pro Arg Ala Arg Thr Val	865	870	875	880
30	Asp Leu Pro Thr Tyr Pro Phe Gln His Gln His Tyr Trp Met Met Asn	885	890	895	
	Thr Gly Ser Ala Ala Glu Pro Ala Glu Leu Gly Leu Gly Asp Ala Arg	900	905	910	
35	His Pro Leu Leu Gly Ser Val Val Thr Val Ala Gly Asp Asp Lys Val	915	920	925	
	Val Phe Ala Gly Arg Leu Ala Leu Arg Thr His Pro Trp Leu Ala Asp	930	935	940	
40	His Thr Val Leu Asp Ala Val Leu Leu Pro Ala Thr Ala Phe Leu Glu	945	950	955	960
	Leu Ala Val Arg Ala Gly Glu Glu Val Ser Cys Pro Val Val His Asp	965	970	975	
45	Leu Thr Leu His Arg Pro Leu Val Val Pro Glu Arg Gly Ala Val Gln	980	985	990	
	Val Gln Met Ala Val Gly Ala Pro Glu Ala Asp Gly Arg Arg Glu Val	995	1000	1005	
50	Arg Val Tyr Ser Arg Pro Asp Asp Ala Glu His Glu Trp Thr Leu	1010	1015	1020	
	His Ala Ala Gly Leu Leu Ala Ser Ala Ala Thr Ala Glu Pro Ala Val	1025	1030	1035	1040

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	Ala Ala Gly Ala Trp Pro Pro Pro Glu Ala Gln Ala Val Asp Leu Asp	1045	1050	1055
5	Gly Phe Tyr Ala Gly Leu Ala Glu His Gly Tyr His Tyr Gly Pro Leu	1060	1065	1070
	Phe Gln Gly Val Arg Ala Ala Trp Arg Leu Gly Asp Asp Val Leu Ala	1075	1080	1085
10	Glu Ile Val Leu Pro Glu Ala Ala Gly Ala Asp Ala Ala Arg Tyr Gly	1090	1095	1100
	Met His Pro Ala Leu Leu Asp Ala Val Leu His Ala Ala Arg Leu Gly	1105	1110	1115
15	Ala Phe Arg Glu Arg Ser Glu Glu Lys Tyr Leu Pro Phe Ala Trp Glu	1125	1130	1135
	Gly Val Thr Leu Arg Thr Arg Gly Ala Thr Ala Val Arg Ala Arg Ile	1140	1145	1150
20	Ser Arg Ala Gly Thr Asp Ala Ile Arg Leu Asp Val Thr Asp Thr Ala	1155	1160	1165
	Asp Arg Pro Val Leu Thr Ala Glu Ser Leu Thr Leu Arg Pro Val Ser	1170	1175	1180
25	Ala Gly Gln Leu Met Ala Val Pro Arg Asp Ser Leu Phe Arg Val Asp	1185	1190	1195
	Trp Val Ser Ala Pro Ala Ala Asn Gly Pro Gly Leu Arg Leu Ala Arg	1205	1210	1215
30	Ala Ala Thr Val Glu Ala Ala Leu Ala Ala Asp Ala Asp Ile Val Val	1220	1225	1230
	Val Pro Cys Leu Asp Ser Glu Gly Pro His Gln Ala Thr Tyr Gln Ala	1235	1240	1245
35	Leu Glu Leu Leu Gln Arg Trp Leu Ala Ala Asp Thr Gly Thr Thr Thr	1250	1255	1260
	Leu Ala Leu Leu Thr His Arg Ala Val Ala Val Gly Asp Asp Val His	1265	1270	1275
40	Asp Leu His His Ala Pro Leu Trp Gly Leu Val Arg Thr Ala Gln Thr	1285	1290	1295
	Glu His Pro Gly Cys Phe Arg Leu Val Asp Ser Asp Asp Pro Asp Pro	1300	1305	1310
45	Thr Thr Asp Val Leu Ala Ala Ala Leu Ala Thr Gly Glu Pro Gln Val	1315	1320	1325
	Ala Ile Arg Asp Gly Ala Val Leu Ala Pro Arg Leu Thr Ala Ala Ser	1330	1335	1340
50	Ala Pro Arg Glu Pro Ala Glu Trp Asp Ala Glu Gly Thr Val Leu Ile	1345	1350	1355
	Thr Gly Gly Ser Gly Ala Leu Ala Gly Ile Val Ala Gln His Leu Val	1365	1370	1375
55	Ala Arg His Gly Val Arg Arg Leu Val Leu Ala Ser Arg Ser Gly Arg			

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	1380	1385	1390
5	Pro Ala Pro Gly Ala Asp Leu Leu Asp Ala Asp Val Thr Ala Val Ser 1395 1400 1405		
	Cys Asp Val Ser Asp Arg Asp Ala Val Ala Ala Leu Leu Ala Ser Val 1410 1415 1420		
10	Pro Asp Glu His Pro Leu Thr Ala Val Val His Thr Ala Gly Val Leu 1425 1430 1435 1440		
	Asp Asp Gly Val Leu His Ala Leu Thr Thr Glu Arg Ile Asp Thr Ser 1445 1450 1455		
15	Phe Ala Ala Lys Val Asp Gly Ala Arg His Leu His Glu Leu Thr Ser 1460 1465 1470		
	His Leu Asp Leu Thr Ala Phe Val Leu Phe Ser Ser Ala Ser Ala Val 1475 1480 1485		
20	Leu Gly Ala Ala Gly Gln Gly Asn Tyr Ala Ala Ala Asn Ala Tyr Leu 1490 1495 1500		
	Asp Ala Leu Ala Ala His Arg Arg Ser Asn Asp Leu Pro Ala Val Ser 1505 1510 1515 1520		
25	Leu Ala Trp Gly Leu Trp Ala Glu His Glu Gly Met Ala Arg Gly Leu 1525 1530 1535		
	Gly Asp Ala Glu Leu Thr Arg Ile Ser Arg Ile Gly Val Thr Ala Leu 1540 1545 1550		
30	Ser Ala Glu Asp Gly Met Arg Leu Phe Asp Ala Gly Cys Ala Gly Asp 1555 1560 1565		
	Gln Ser Gln Leu Val Pro Met Arg Val Asp Thr Ala Ala Leu Arg Ala 1570 1575 1580		
35	Arg Arg Asp His Leu Pro Ala Pro Met Trp Ser Leu Val Pro Glu Arg 1585 1590 1595 1600		
	Thr Arg Ala Ala Arg Thr Gln Pro Ala Ala Ser Leu Arg Asp Arg Leu 1605 1610 1615		
40	Ala Glu Leu Thr Ala Pro Glu Arg Lys Arg Thr Val Leu Asn Leu Val 1620 1625 1630		
	Arg Asn Ala Val Ala Asp Thr Leu Gly His Asn Ala Ala Asp Gly Val 1635 1640 1645		
45	Pro Pro Asp Gln Ser Leu Asp Ala Ala Gly Phe Asp Ser Leu Thr Ala 1650 1655 1660		
	Val Glu Phe Arg Asn Arg Leu Ser Ala Val Thr Asp Leu Arg Leu Pro 1665 1670 1675 1680		
50	Ala Thr Leu Thr Tyr Asp His Pro Thr Pro Ala Ala Ile Ala Glu His 1685 1690 1695		
	Ile Leu Thr Arg Leu Thr Leu Leu Lys Glu Thr Ala Ala Pro Ala Val 1700 1705 1710		
55	Gly Thr Ala Pro Val Ala Ala Pro Thr Glu Asp Asp Ala Ile Val Ile 1715 1720 1725		

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	Val Gly Met Ala Gly Arg Phe Pro Gly Gly Val Arg Thr Pro Glu Gly	
	1730	1735 1740
5	Leu Trp Asp Leu Val His Ser Gly Thr Asp Ala Ile Ser Glu Trp Pro	
	1745	1750 1755 1760
	Thr Asp Arg Gly Trp Asp Val Glu Asn Leu Tyr Asp Pro Asp Pro Asp	
		1765 1770 1775
10	Ala Val Gly Lys Ser Tyr Val Arg His Gly Gly Phe Leu His Asp Val	
		1780 1785 1790
	Ala Gly Phe Asp Ala Gly Phe Phe Gly Ile Ser Pro Arg Glu Ala Leu	
		1795 1800 1805
15	Ala Met Asp Pro Gln Gln Arg Leu Leu Leu Glu Cys Ser Tyr Glu Ala	
		1810 1815 1820
	Leu Glu Arg Ala Gly Ile Asp Pro Ala Thr Leu Arg Gly Ser Arg Ser	
		1825 1830 1835 1840
20	Gly Val Tyr Ala Gly Val Met Tyr His Glu Tyr Ala Ser Arg Leu Gly	
		1845 1850 1855
	Ala Thr Pro Ala Gly Phe Glu Gly Thr Leu Gly Thr Gly Ser Ser Gly	
		1860 1865 1870
25	Ser Ile Ala Ser Gly Arg Ile Ser Tyr Thr Phe Asp Leu Thr Gly Pro	
		1875 1880 1885
	Ala Val Thr Val Asp Thr Ala Cys Ser Thr Ser Leu Val Gly Leu His	
		1890 1895 1900
30	Leu Ala Val Gln Ala Leu Arg Ala Gly Glu Cys Glu Leu Ala Leu Ala	
		1905 1910 1915 1920
	Gly Gly Val Thr Val Met His Thr Pro Arg Pro Phe Val Glu Phe Ser	
		1925 1930 1935
35	Arg Gln Arg Gly Leu Ala Ala Asp Gly Arg Ser Lys Ala Phe Ala Ala	
		1940 1945 1950
	Ser Ala Asp Gly Val Ala Trp Ala Glu Gly Ala Gly Ile Leu Val Leu	
		1955 1960 1965
40	Glu Arg Leu Ser Ala Ala Arg Arg Asn Gly His Arg Val Leu Ala Val	
		1970 1975 1980
	Val Arg Gly Ser Ala Val Asn Gln Asp Gly Ala Ser Asn Gly Leu Thr	
		1985 1990 1995 2000
45	Ala Pro Asn Gly Pro Ser Gln Gln Arg Val Ile Arg Ala Ala Leu Ala	
		2005 2010 2015
	Ser Ala Gly Leu Gly Pro Ala Asp Val Asp Val Val Glu Ala His Gly	
		2020 2025 2030
50	Thr Gly Thr Ala Leu Gly Asp Pro Ile Glu Ala Gln Ala Leu Leu Ala	
		2035 2040 2045
55	Thr Tyr Gly Arg Gly Arg Asp Ala Asp Arg Pro Leu Trp Leu Gly Ser	
		2050 2055 2060

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	Val Lys Ser Asn Ile Gly His Thr Gln Ala Ala Ala Gly Val Ala Ser	2065	2070	2075	2080
5	Val Ile Lys Met Val Gln Ala Met Gln Ala Gly Val Leu Pro Arg Thr	2085	2090	2095	
	Leu His Val Asp Glu Pro Ser Gly Glu Val Asp Trp Asp Ser Gly Ala	2100	2105	2110	
10	Val Arg Leu Leu Thr Glu Ala Arg Glu Trp Pro Ser Gly Glu Gly Arg	2115	2120	2125	
	Val Arg Arg Ala Gly Val Ser Ser Phe Gly Ile Ser Gly Thr Asn Ala	2130	2135	2140	
15	His Val Ile Leu Glu Glu Pro Pro Ala Glu Asp Ala Leu Pro Glu Pro	2145	2150	2155	2160
	Glu Ala Gly Asp Val Val Pro Trp Val Leu Ser Ala Arg Ser Ala Glu	2165	2170	2175	
20	Ala Leu Arg Glu Gln Ala Ala Arg Leu Ala Ser Val Ala Gly Gly Leu	2180	2185	2190	
	Asn Val Val Asp Val Gly Trp Ser Leu Ala Ser Thr Arg Ala Ala Phe	2195	2200	2205	
25	Glu His Arg Ala Val Val Val Gly Gly Asp Arg Glu Glu Leu Leu Gly	2210	2215	2220	
	Lys Leu Ser Ser Val Ser Gly Val Glu Val Gly Val Gly Val Gly Ala	2225	2230	2235	2240
30	Gly Gly Gly Val Val Leu Val Phe Ala Gly Gln Gly Cys Gln Trp Val	2245	2250	2255	
	Gly Met Gly Arg Glu Leu Leu Gly Ser Ser Leu Val Phe Ala Glu Ser	2260	2265	2270	
35	Met Arg Glu Cys Ala Ala Ala Leu Ser Pro Phe Val Asp Phe Ser Val	2275	2280	2285	
	Val Asp Val Leu Gly Ser Ala Gly Glu Leu Gly Arg Val Glu Val Val	2290	2295	2300	
40	Gln Pro Ala Leu Trp Ala Val Met Val Ser Leu Ala Arg Val Trp Arg	2305	2310	2315	2320
	Ser Trp Gly Val Pro Val Ala Ala Val Val Gly His Ser Gln Gly Glu	2325	2330	2335	
45	Ile Ala Ala Ala Thr Val Ala Gly Ala Leu Ser Val Gly Asp Ala Ala	2340	2345	2350	
	Arg Val Val Ala Leu Arg Ser Arg Leu Ile Ala Glu Arg Leu Ser Gly	2355	2360	2365	
50	Leu Gly Gly Met Val Ser Val Ala Leu Ser Arg Glu Arg Val Val Ser	2370	2375	2380	
	Leu Ile Ala Gly Val Pro Gly Val Ser Val Ala Ala Val Asn Gly Ser	2385	2390	2395	2400
55	Ser Ser Thr Val Val Ser Gly Glu Ala Ala Gly Leu Glu Arg Val Leu				

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	2405	2410	2415
	Ala Ala Cys Val Ser Ser Gly Val Arg Ala Arg Arg Ile Asp Val Asp		
	2420	2425	2430
5	Tyr Ala Ser His Ser Val Gln Val Glu Leu Ile Arg Glu Glu Leu Leu		
	2435	2440	2445
	Gly Val Leu Asp Gly Ile Val Pro Arg Ser Gly Glu Ile Pro Phe Val		
10	2450	2455	2460
	Ser Thr Val Thr Gly Glu Arg Ile Asp Thr Val Glu Leu Gly Ala Glu		
	2465	2470	2475
	Tyr Trp Tyr Arg Asn Leu Arg Gln Thr Val Glu Phe Gln Ser Val Val		
15	2485	2490	2495
	Glu Gly Leu Val Ala Gln Gly Cys Arg Val Phe Leu Glu Ser Ser Pro		
	2500	2505	2510
	His Pro Val Leu Thr Val Gly Ile Glu Glu Ser Ala Asp Arg Val Val		
20	2515	2520	2525
	Ala Leu Glu Ser Leu Arg Arg Gly Glu Gly Gly Leu Arg Arg Leu Val		
	2530	2535	2540
	Asp Ala Ala Gly Glu Ala Trp Val Arg Gly Val Pro Ile Asp Trp Ala		
25	2545	2550	2555
	Gly Met Leu Ala Gly Gly Arg Arg Val Asp Leu Pro Thr Tyr Pro Phe		
	2565	2570	2575
	Gln His Gln Pro Tyr Trp Leu Asp Ser Pro Arg His Pro Ala Gly Asp		
30	2580	2585	2590
	Val Thr Ala Val Gly Leu Thr Glu Ala Gly His Ala Phe Val Pro Ala		
	2595	2600	2605
	Ala Val Asp Leu Pro Asp Gly Gln Arg Val Trp Thr Gly Arg Leu Ser		
35	2610	2615	2620
	Leu Pro Ser Tyr Pro Trp Leu Ala Asp His Gln Val Leu Gly Gln Val		
	2625	2630	2635
	Leu Leu Pro Gly Val Val Trp Val Glu Leu Ala Leu His Ala Gly His		
40	2645	2650	2655
	Gln Ala Gly Cys Asp Ser Val Asp Glu Leu Thr Leu Gln Ser Pro Leu		
	2660	2665	2670
	Val Leu Gly Ala Ser Asp Thr Val Gln Val Arg Val Val Val Thr Glu		
45	2675	2680	2685
	Thr Glu Glu Pro Gly Thr Arg Thr Val Ser Met His Ser Arg Arg Asp		
	2690	2695	2700
	Asp Gly Ser Trp Val Thr His Ala Glu Gly Ile Leu Gly Ala Gly Gly		
50	2705	2710	2715
	Pro Pro Pro Glu Pro Leu Pro Glu Trp Pro Pro Thr Gly Ala Met Pro		
	2725	2730	2735
	Leu Asp Val Glu Gly Phe Tyr Asp Glu Leu Ala Ala Gly Gly Tyr His		
55	2740	2745	2750

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	Tyr Gly Pro Gln Phe Arg Cys Leu Arg Arg Ala Trp Arg Ala Gly Glu	2755	2760	2765
5	Asp Leu Val Ala Glu Ile Ser Leu Pro Glu Gly Thr Asp Val Asp Ala	2770	2775	2780
	Tyr Gly Leu His Pro Gly Leu Phe Asp Ala Ala Val His Ser Val Ala	2785	2790	2795
10	Cys Ala Arg Thr Ser Ala Gly Ala Gly Asp Asp Gly Pro Arg Leu Pro	2805	2810	2815
	Phe Ala Phe Ser Asp Val Arg Leu Phe Ala Thr Gly Val Thr Ser Leu	2820	2825	2830
15	Arg Val Arg Ile Asp Pro Gln Asn Ser Ser Trp Gln Ala Trp Asp Glu	2835	2840	2845
	Ser Gly Leu Pro Val Leu Thr Ile Gly Arg Leu Ala Gly Arg Pro Val	2850	2855	2860
20	Asp Ala Asp Gln Phe Ala Val Arg Arg Ala Gly His Leu Phe Arg Val	2865	2870	2875
	Glu Thr Arg His Glu Ala Leu Ala Gly Pro Ala Pro Ala Ser Trp Ala	2885	2890	2895
25	Val Ile Gly Ala Asp Pro Ala Gly Tyr Ala Ala Ala Leu Glu Ala Thr	2900	2905	2910
	Gly Ala Gln Val Thr Thr Ala Ala Asp Leu Ala Gly Leu Thr Ser Ala	2915	2920	2925
30	Pro Glu Ala Ala Leu Phe Thr Leu Pro Gly Thr Lys Asp Ala Gly Val	2930	2935	2940
	Thr Glu Glu Val Pro Thr Ala Val Arg Glu Ala Thr Ala Gln Val Leu	2945	2950	2955
35	Glu Val Leu Gln Asp Trp Leu Thr Asp Gly Arg Phe Asp Asp Ala Arg	2965	2970	2975
	Leu Val Val Val Ser Arg Glu Ala Glu Asp Gly Asp Leu Leu His Gly	2980	2985	2990
40	Thr Ala Arg Gly Leu Leu Arg Ala Ala Gln Ala Glu His Pro Asp Arg	2995	3000	3005
	Ile Thr Leu Val Asp Leu Asp Ala His Pro Ala Ser Leu Thr Ala Leu	3005	3015	3020
45	Pro Gly Phe Ala Leu Gly Pro Glu Pro Glu Val Val Val Arg Ala Gly	3025	3030	3035
	Asp Gly Arg Ala Pro Arg Leu Ala Arg Ala Gln Ala Pro Thr Gly Ala	3045	3050	3055
50	Gly Ser Leu Gly Thr Gly Thr Val Leu Ile Thr Gly Gly Thr Gly Thr	3060	3065	3070
	Leu Gly Gly Leu Leu Ala Arg His Leu Val Glu Thr His Gly Val Thr	3075	3080	3085
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	Arg Leu Leu Leu Val Ser Arg Arg Gly Pro Ala Ala Asp Gly Ala Asp	3090	3095	3100
5	Arg Leu His Ala Glu Leu Thr Gly His Gly Ala His Val Asp Ile Val	3105	3110	3115 3120
	Ala Ala Asp Leu Gly Asp Arg Thr Ser Val Ala Ala Leu Leu Ala Thr	3125	3130	3135
10	Val Asp Ala Asp His Pro Leu Ser Ala Val Val His Ala Ala Gly Ala	3140	3145	3150
	Leu Asp Asp Gly Val Leu Gly Thr Arg Ser Ala Asp Trp Leu Asp Pro	3155	3160	3165
15	Val Leu Arg Pro Lys Ala Asp Ala Ala Trp His Leu His Glu Leu Thr	3170	3175	3180
	Ala Glu Leu Pro Leu Thr Ala Phe Val Met Phe Ser Ser Ala Ala Ser	3185	3190	3195 3200
20	Val Leu Gly Ala Ala Gly Gln Ala Asn Tyr Ala Ala Ala Asn Gly Phe	3205	3210	3215
	Leu Asp Ala Leu Ala Ala His Arg Ala Ala Arg Gly Leu Pro Gly Thr	3220	3225	3230
25	Ser Leu Ala Trp Gly Leu Trp Glu His Arg Ser Glu Leu Thr Arg His	3235	3240	3245
	Thr Gly Ser Pro Ser Arg Ser Ile Ala Ala Val Gly Ala Leu Ser Thr	3250	3255	3260
30	Ala Glu Ala Leu Ala Ala Phe Asp Ala Gly Leu Ala Ser Gly Glu Pro	3265	3270	3275 3280
	Leu Ala Val Pro Ile Arg Leu Glu Ser Thr Ser Ser Glu Glu Val Pro	3285	3290	3295
35	Pro Met Leu Arg Gly Leu Val Arg Val Arg Arg Arg Ala Ala Thr Gly	3300	3305	3310
	Thr Glu Pro Ala Ala Ser Ala Gly Ala Ala Gln Glu Val Arg Gln Leu	3315	3320	3325
40	Ala Glu Leu Gly Ala Asp Glu Arg Gln Arg Arg Val Gln Arg Ile Val	3330	3335	3340
	Leu Asp Thr Ala Ala Ala Val Leu Gly His Asp Ser His Asp Ala Ile	3345	3350	3355 3360
45	Pro Leu Thr Arg Gly Phe Leu Glu Leu Gly Phe Asp Ser Leu Thr Ala	3365	3370	3375
	Val Arg Leu Arg Asn Arg Leu Ala Arg Arg Leu Gly Leu Arg Leu Pro	3380	3385	3390
50	Ala Thr Val Val Phe Asp His Pro Ser Pro Ala Ala Leu Ala Ala His	3395	3400	3405
	Leu Val Glu His Leu Val Gly Thr Val Asp Pro Thr Ala Gln Ala Met	3410	3415	3420
55	Glu Gln Leu Glu Ala Leu Arg Arg Ser Val His Ala Ala Thr Pro Ala			

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	3425		3430		3435		3440
5	Gly	Gly	Leu	Asp	Arg	Ala	Leu
				3445			
					3450		
						3455	
	Asp	Glu	Met	Arg	His	Val	Asp
				3460			
					3465		
						3470	
10	Gly	Ser	Gly	Asp	Asp	Leu	Glu
				3475			
					3480		
						3485	
	Leu	Ile	Asp	Asn	Glu	Leu	Gly
				3490			
					3495		
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<211> 1620

<212> PRT

<213> Streptomyces parvulus Tü4055

<400> 5

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	1				5					10					15	
5	Asp	Leu	Arg	Ser	Ala	Arg	Ala	Arg	Leu	Gln	Glu	Leu	Glu	Ser	Ala	Asp
				20					25					30		
	Thr	Asp	Pro	Ile	Ala	Ile	Ile	Gly	Met	Gly	Cys	Arg	Leu	Pro	Gly	Gly
			35					40					45			
10	Val	Arg	Thr	Pro	Glu	Asp	Leu	Trp	Asp	Leu	Val	Glu	Lys	Lys	His	Asp
	50						55					60				
	Ala	Ile	Gly	Pro	Phe	Pro	Ala	Asp	Arg	Gly	Trp	Asp	Leu	Glu	Asn	Leu
15	65					70				75						80
	Tyr	Asp	Pro	Asp	Pro	Asp	Ala	Pro	Gly	Lys	Ala	Tyr	Val	Arg	Glu	Gly
					85					90					95	
	Gly	Phe	Val	His	Asp	Val	Ala	Gly	Phe	Asp	Ala	Gly	Phe	Phe	Gly	Ile
20				100				105						110		
	Ser	Pro	Arg	Glu	Ala	Leu	Ala	Met	Asp	Pro	Gln	His	Arg	Leu	Leu	Leu
			115					120					125			
25	Glu	Cys	Ser	Trp	Glu	Ala	Leu	Glu	Arg	Ala	Gly	Ile	Asp	Pro	Ser	Ser
	130						135					140				
	Leu	Glu	Gly	Thr	Arg	Thr	Gly	Val	Tyr	Thr	Gly	Leu	Met	Thr	His	Glu
	145					150					155					160
30	Tyr	Ala	Thr	Arg	Leu	Pro	Ser	Ile	Asp	Glu	Glu	Leu	Glu	Gly	Val	Ile
					165					170					175	
	Gly	Ile	Gly	Asn	Ala	Gly	Ser	Val	Ala	Ser	Gly	Arg	Val	Ser	Tyr	Thr
35				180					185					190		
	Leu	Gly	Leu	Asn	Gly	Pro	Ala	Val	Thr	Val	Asp	Thr	Ala	Cys	Ser	Ser
			195					200					205			
	Ser	Leu	Val	Ala	Leu	His	Leu	Ala	Ala	Gln	Ala	Leu	Arg	Gln	Gly	Gln
40	210						215					220				
	Cys	Thr	Leu	Ala	Leu	Ala	Gly	Gly	Ala	Ser	Val	Ile	Ala	Ala	Pro	Thr

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				225					230						235				240
				Val	Phe	Ala	Thr	Phe	Ser	Arg	Gln	Arg	Gly	Leu	Ala	Pro	Asp	Gly	Arg
								245					250					255	
5				Cys	Lys	Ala	Phe	Ser	Ser	Thr	Thr	Asp	Gly	Thr	Gly	Phe	Gly	Glu	Gly
							260					265					270		
				Val	Gly	Val	Leu	Val	Leu	Glu	Arg	Leu	Ser	Asp	Ala	Arg	Arg	Asn	Gly
10						275					280					285			
				His	Glu	Val	Leu	Ala	Val	Val	Arg	Gly	Ser	Ala	Val	Asn	Gln	Asp	Gly
					290					295					300				
				Ala	Ser	Ser	Gly	Phe	Thr	Ala	Pro	Asn	Gly	Pro	Ser	Gln	Gln	Asp	Val
15									310					315					320
				Ile	Arg	Glu	Ala	Leu	Ala	Asp	Gly	Arg	Leu	Thr	Pro	Ala	Asp	Val	Asp
								325					330					335	
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20							340					345					350		
				Ala	Gln	Ala	Leu	Leu	Ala	Thr	Tyr	Gly	Arg	Gly	Arg	Asp	Ala	Asp	Arg
						355					360					365			
				Pro	Leu	Trp	Leu	Gly	Ser	Val	Lys	Ser	Asn	Ile	Gly	His	Thr	Gln	Ala
25					370					375					380				
				Ala	Ala	Gly	Val	Ala	Ser	Val	Ile	Lys	Met	Val	Gln	Ala	Met	Gln	Ala
					385					390				395					400
				Gly	Val	Leu	Pro	Arg	Thr	Leu	His	Val	Asp	Glu	Pro	Ser	Gly	Glu	Val
30							405						410					415	
				Asp	Trp	Asp	Ser	Gly	Ala	Val	Arg	Leu	Leu	Thr	Glu	Ala	Arg	Glu	Trp
							420					425					430		
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35						435					440					445			
				Ile	Ser	Gly	Thr	Asn	Ala	His	Val	Ile	Leu	Glu	Glu	Pro	Pro	Ala	Glu
					450					455					460				
				Asp	Ala	Leu	Pro	Glu	Pro	Glu	Ala	Gly	Asp	Val	Val	Pro	Trp	Val	Leu
40									470					475					480
				Ser	Ala	Arg	Ser	Ala	Glu	Ala	Leu	Arg	Glu	Gln	Ala	Ala	Arg	Leu	Ala
								485					490					495	
				Ser	Val	Ala	Gly	Gly	Leu	Asn	Val	Val	Asp	Val	Gly	Trp	Ser	Leu	Ala
45							500					505					510		
				Ser	Thr	Arg	Ala	Ala	Phe	Glu	His	Arg	Ala	Val	Val	Val	Gly	Gly	Asp
						515					520					525			
				Arg	Glu	Glu	Leu	Leu	Gly	Lys	Leu	Ser	Ser	Val	Ser	Gly	Val	Glu	Val
50										535					540				
				Gly	Val	Gly	Val	Gly	Ala	Gly	Gly	Gly	Val	Val	Leu	Val	Phe	Ala	Gly
									550					555					560
				Gln	Gly	Cys	Gln	Trp	Val	Gly	Met	Gly	Arg	Glu	Leu	Leu	Gly	Ser	Ser
55								565					570					575	

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	Leu	Val	Phe	Ala	Glu	Ser	Met	Arg	Glu	Cys	Ala	Ala	Ala	Leu	Ser	Pro	
				580					585					590			
5	Phe	Val	Asp	Phe	Ser	Val	Val	Asp	Val	Leu	Gly	Ser	Ala	Gly	Glu	Leu	
			595					600					605				
	Gly	Arg	Val	Glu	Val	Val	Gln	Pro	Ala	Leu	Trp	Ala	Val	Met	Val	Ser	
		610					615					620					
10	Leu	Ala	Arg	Val	Trp	Arg	Ser	Trp	Gly	Val	Pro	Val	Ala	Ala	Val	Val	
	625					630					635					640	
	Gly	His	Ser	Gln	Gly	Glu	Ile	Ala	Ala	Ala	Thr	Val	Ala	Gly	Ala	Leu	
				645						650					655		
15	Ser	Val	Gly	Asp	Ala	Ala	Arg	Val	Val	Ala	Leu	Arg	Ser	Arg	Leu	Ile	
				660					665					670			
	Ala	Glu	Arg	Leu	Ser	Gly	Leu	Gly	Gly	Met	Val	Ser	Val	Ala	Leu	Ser	
			675					680					685				
20	Arg	Glu	Arg	Val	Val	Ser	Leu	Ile	Ala	Gly	Val	Pro	Gly	Val	Ser	Val	
		690					695					700					
	Ala	Ala	Val	Asn	Gly	Ser	Ser	Ser	Thr	Val	Val	Ser	Gly	Glu	Ala	Ala	
	705				710						715					720	
25	Gly	Leu	Glu	Arg	Val	Leu	Ala	Ala	Cys	Val	Ser	Ser	Gly	Val	Arg	Ala	
				725					730					735			
	Arg	Arg	Ile	Asp	Val	Asp	Tyr	Ala	Ser	His	Ser	Val	Gln	Val	Glu	Leu	
				740				745					750				
30	Ile	Arg	Glu	Glu	Leu	Leu	Gly	Val	Leu	Asp	Gly	Ile	Val	Pro	Arg	Ser	
			755				760						765				
	Gly	Glu	Ile	Pro	Phe	Val	Ser	Thr	Val	Thr	Gly	Glu	Arg	Ile	Asp	Thr	
		770					775					780					
35	Val	Glu	Leu	Gly	Ala	Glu	Tyr	Trp	Tyr	Arg	Asn	Leu	Arg	Gln	Thr	Val	
	785					790					795					800	
	Glu	Phe	Gln	Ser	Val	Val	Glu	Gly	Leu	Val	Ala	Gln	Gly	Cys	Arg	Val	
					805					810					815		
40	Phe	Leu	Glu	Ser	Ser	Pro	His	Pro	Val	Leu	Thr	Val	Gly	Ile	Glu	Glu	
				820					825					830			
	Ser	Ala	Asp	Arg	Val	Val	Ala	Leu	Glu	Ser	Leu	Arg	Arg	Gly	Glu	Gly	
			835					840					845				
45	Gly	Leu	Arg	Arg	Leu	Val	Asp	Ala	Ala	Gly	Glu	Ala	Trp	Val	Arg	Gly	
		850					855					860					
	Val	Pro	Ile	Asp	Trp	Ala	Gly	Met	Leu	Ala	Gly	Gly	Arg	Arg	Val	Asp	
	865					870					875					880	
50	Leu	Pro	Thr	Tyr	Pro	Phe	Gln	His	Gln	Pro	Tyr	Trp	Leu	Asp	Ser	Pro	
					885					890					895		
55	Arg	His	Pro	Ala	Gly	Asp	Val	Thr	Gly	Pro	Gly	Asp	Asp	Glu	Phe	Trp	
				900					905					910			

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	Ala	Ala	Val	Glu	His	Gly	Glu	Ala	Thr	Glu	Leu	Ala	Asp	Leu	Leu	Arg	
			915					920					925				
5	Arg	Ser	Ala	Ala	Glu	Pro	Gly	Gln	Asp	Leu	His	Ala	Pro	Val	Ala	Ala	
			930				935					940					
	Leu	Leu	Pro	Thr	Leu	Ala	Thr	Trp	Arg	Arg	Asp	Arg	Gln	Arg	Arg	Ala	
	945					950					955					960	
10	Ala	Val	Asp	Ser	Trp	Arg	Tyr	Arg	Ile	Val	Trp	Arg	Pro	Val	Ala	Thr	
					965					970					975		
	Pro	Ser	Tyr	Asp	Arg	Val	Leu	Ser	Gly	Arg	Trp	Ala	Val	Val	Val	Pro	
				980					985					990			
15	Ala	Gly	His	Glu	Asp	Asp	Pro	Val	Val	Asp	Trp	Val	Cys	Ser	Ala	Leu	
			995				1000						1005				
	Arg	Asp	His	Gly	Gly	Glu	Pro	Glu	Arg	Met	Val	Leu	Gly	Pro	Arg	Glu	
	1010					1015						1020					
20	Ser	Arg	Ser	Ala	Leu	Ala	Thr	Arg	Leu	Ala	Ala	Asp	Pro	Pro	Gly	Gly	
	1025				1030						1035					1040	
	Val	Val	Ser	Leu	Leu	Gly	Leu	Ser	Gly	Ala	Ala	His	Pro	Asp	His	Glu	
				1045					1050					1055			
25	Val	Leu	Pro	Ser	Ala	Val	Ala	Gly	Thr	Val	Leu	Leu	Ala	Gln	Ala	Leu	
				1060				1065						1070			
	Ser	Asp	Gly	Ala	Val	Arg	Ala	Pro	Val	Trp	Thr	Leu	Thr	Arg	Asn	Gly	
		1075					1080					1085					
30	Val	Ser	Ala	Thr	Ala	Thr	Asp	Pro	Val	Ala	Pro	Thr	His	Ala	Ala	Gln	
	1090					1095						1100					
	Val	Trp	Ala	Val	Ala	Arg	Val	Ala	Gly	Leu	Glu	His	Pro	Glu	Ala	Trp	
	1105				1110					1115					1120		
35	Gly	Gly	Leu	Leu	Asp	Leu	Pro	Asp	Arg	Leu	Asp	Asp	Arg	Ala	Ala	Ala	
				1125					1130					1135			
	Arg	Phe	Ala	Ala	Val	Leu	Ser	Ala	Gly	Glu	Asp	Glu	Asp	Gln	Leu	Ala	
			1140					1145					1150				
40	Leu	Arg	Asp	Ala	Gly	Leu	Leu	Ala	Arg	Arg	Leu	Val	Arg	Ala	Pro	Val	
		1155					1160					1165					
	Pro	Arg	Asp	Ala	Val	Thr	Ala	Gly	Trp	Gln	Pro	Arg	Asp	Thr	Ala	Leu	
	1170				1175							1180					
45	Val	Thr	Gly	Gly	Thr	Gly	Gly	Leu	Gly	Gly	Gln	Val	Ala	Arg	Trp	Leu	
	1185				1190					1195					1200		
	Ala	Ala	Ala	Gly	Val	Arg	His	Leu	Val	Leu	Val	Ser	Arg	Arg	Gly	Ala	
				1205					1210				1215				
50	Glu	Ala	Glu	Gly	Ala	Asp	Arg	Leu	Arg	Asp	Asp	Leu	Thr	Ala	Leu	Gly	
			1220					1225				1230					
	Val	Gln	Val	Thr	Phe	Gly	Ala	Cys	Asp	Val	Ala	Asp	Arg	Ala	Ala	Leu	
		1235				1240						1245					
55	Ser	Ala	Leu	Leu	Asp	Arg	Val	Gln	Glu	Asp	Gly	Pro	Pro	Ile	Arg	Thr	

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5	Val Val His Ala Ala Gly Ser Gly Arg Ala Ala Arg Leu Leu Asp Thr 1265 1270 1275 1280		
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10	Arg Asn Leu His Glu Leu Leu Asp Asp Val Asp Ala Phe Val Leu Phe 1300 1305 1310		
	Ser Ser Gly Ala Gly Val Trp Gly Ser Ser Ala Gln Gly Ala Tyr Ala 1315 1320 1325		
15	Ala Ala Asn Ala Tyr Leu Asp Ala Leu Ala Glu Gln Arg Arg Gly Gln 1330 1335 1340		
	Gly Arg Pro Ala Thr Ser Val Ala Trp Gly Ala Trp Ala Gly Asp Gly 1345 1350 1355 1360		
20	Met Thr Ala Ala Ala Gly Glu Glu Trp Trp Ser Arg Gln Gly Leu Arg 1365 1370 1375		
	Phe Met Ala Pro Glu Ala Ala Leu Asp Ala Leu Arg Gln Ala Val Asp 1380 1385 1390		
25	Arg Ala Glu Ser Thr Leu Val Val Ala Asp Ile Asp Trp Lys Thr Phe 1395 1400 1405		
	Ala Pro Leu Phe Thr Ser Ala Arg Ser Arg Pro Leu Ile Thr Asp Ile 1410 1415 1420		
30	Pro Glu Ala Arg Pro Glu Pro Arg Pro Glu Gly Ala Asp Gln Pro Thr 1425 1430 1435 1440		
	Gln Gly Leu Val Ala Lys Leu Ala Val Leu Ser Ala Asp Glu Arg Arg 1445 1450 1455		
35	Arg Ala Leu Leu Ala Glu Val Arg Ala Gln Ala Ala Val Val Leu Gly 1460 1465 1470		
	His Pro Gly Ala Asp Ala Val Pro Val Asp Arg Pro Phe Arg Glu Leu 1475 1480 1485		
40	Gly Phe Asp Ser Leu Ser Ala Val Lys Leu Arg Asn Arg Ile Val Ala 1490 1495 1500		
	Ala Thr Gly Leu Glu Leu Pro Ala Thr Leu Val Phe Asp His Pro Thr 1505 1510 1515 1520		
45	Ser Thr Ala Leu Ala Ala Tyr Leu Gly Ala Arg Leu Gly Ile Asp Gly 1525 1530 1535		
	Ala Pro Ala Gly Ser Thr Leu Leu Glu Asp Leu Ala Arg Leu Glu Ser 1540 1545 1550		
50	Thr Val Ala Thr Leu Thr Ala Ala Pro Leu Ala Glu Thr Val Pro Asp 1555 1560 1565		
	Ala Arg Asp Arg Ala Ala Leu Thr Thr Arg Leu Arg Ala Leu Leu Glu 1570 1575 1580		
55	Arg Trp Asp Gln Ala Asp Gly Glu Asp Gln Ala Ala Ala Arg Glu Glu 1585 1590 1595 1600		

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Leu Asp Asp Leu Ser Asp Asp Asp Leu Phe Asp Phe Ile Asp Ala Lys
1605 1610 1615

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Phe Gly Arg Ser
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5	Asp	Leu	Ala	Asp	Val	Thr	Glu	Arg	Leu	Gln	Arg	Ala	Glu	Asp	Lys	Asn	
				20					25					30			
	Ala	Glu	Pro	Ile	Ala	Ile	Val	Gly	Met	Gly	Cys	Arg	Tyr	Pro	Gly	Gly	
			35					40					45				
10	Val	Arg	Ser	Pro	Glu	Glu	Phe	Trp	Asn	Leu	Leu	Asp	Glu	Gly	Val	Asp	
		50					55					60					
	Ala	Val	Ala	Gly	Phe	Pro	Glu	Asp	Arg	Gly	Trp	Asp	Leu	Glu	Asn	Leu	
15		65				70					75					80	
	Tyr	Asp	Pro	Asp	Pro	Asp	Glu	Pro	Gly	Lys	Cys	Tyr	Ala	Arg	Glu	Gly	
					85					90					95		
	Gly	Phe	Leu	Tyr	Asp	Ala	Gly	Glu	Phe	Asp	Ala	Ala	Phe	Phe	Gly	Ile	
20				100					105					110			
	Ser	Pro	Arg	Glu	Ala	Leu	Ser	Met	Asp	Pro	Gln	Gln	Arg	Leu	Leu	Leu	
			115					120					125				
25	Glu	Cys	Ser	Trp	Ser	Ala	Leu	Glu	Arg	Ala	Gly	Ile	Asp	Pro	Gly	Ser	
		130					135					140					
	Leu	Arg	Gly	Lys	Asp	Val	Gly	Val	Tyr	Val	Gly	Ala	Trp	Asn	Ser	Asn	
	145					150					155					160	
30	Tyr	Gly	Arg	Gly	Gly	Gly	Ala	Glu	Ser	Ser	Glu	Gly	His	Leu	Leu	Thr	
				165						170					175		
	Gly	Asn	Ala	Ser	Ser	Val	Val	Ser	Gly	Arg	Val	Ala	Tyr	Val	Leu	Gly	
35				180					185					190			
	Leu	Glu	Gly	Pro	Ala	Val	Thr	Ile	Asp	Thr	Ala	Cys	Ser	Ser	Ser	Leu	
			195					200					205				
40	Val	Gly	Leu	His	Leu	Ala	Ala	Gln	Ala	Leu	Arg	Ser	Gly	Glu	Cys	Gly	
		210					215					220					
	Leu	Ala	Leu	Ala	Gly	Gly	Val	Thr	Val	Met	Ser	Thr	Pro	Leu	Ser	Leu	
	225					230					235					240	
45	Val	Ser	Phe	Ser	Arg	Gln	Arg	Gly	Leu	Ala	Gln	Asp	Gly	Arg	Ser	Lys	
					245					250					255		
	Ala	Phe	Ser	Ala	Asp	Ala	Asp	Gly	Met	Gly	Met	Ala	Glu	Gly	Val	Gly	
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	Val	Leu	Val	Leu	Glu	Arg	Leu	Ser	Glu	Ala	Arg	Arg	Asn	Gly	His	Glu	
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5	Val	Leu	Ala	Val	Leu	Arg	Ser	Ser	Ala	Val	Asn	Gln	Asp	Gly	Ala	Ser	
			290					295				300					
	Asn	Gly	Leu	Ser	Ala	Pro	Asn	Gly	Pro	Ala	Gln	Gln	Arg	Val	Ile	Gln	
		305				310					315					320	
10	Ser	Ala	Leu	Thr	Val	Gly	Arg	Leu	Ala	Pro	Ser	Asp	Ile	Asp	Val	Val	
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	Glu	Ala	His	Gly	Thr	Gly	Thr	Ala	Leu	Gly	Asp	Pro	Ile	Glu	Ala	Gln	
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			355					360					365				
	Trp	Leu	Gly	Ser	Val	Lys	Ser	Asn	Ile	Gly	His	Thr	Gln	Ala	Ala	Ala	
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	Leu	Pro	Arg	Thr	Leu	His	Val	Asp	Glu	Pro	Thr	Gly	Glu	Val	Asp	Trp	
				405						410					415		
25	Asp	Ser	Gly	Ala	Val	Arg	Leu	Leu	Thr	Glu	Ala	Arg	Glu	Trp	Pro	Ser	
				420					425					430			
	Gly	Glu	Gly	Arg	Val	Arg	Arg	Ala	Gly	Val	Ser	Ser	Phe	Gly	Ile	Ser	
			435					440					445				
30	Gly	Thr	Asn	Ala	His	Val	Ile	Val	Glu	Glu	Ala	Pro	Glu	Glu	Glu	Pro	
		450					455					460					
	Arg	Pro	Glu	Ala	Pro	Ser	Val	Asp	Val	Val	Pro	Trp	Val	Leu	Ser	Ala	
		465				470					475					480	
35	Arg	Ser	Ala	Glu	Ala	Leu	Arg	Glu	Gln	Ala	Ala	Arg	Leu	Ala	Ser	Val	
				485						490					495		
	Ala	Gly	Gly	Leu	Asn	Val	Val	Asp	Val	Gly	Trp	Ser	Leu	Ala	Ser	Thr	
			500						505					510			
40	Arg	Ala	Ala	Phe	Glu	His	Arg	Ala	Val	Val	Val	Gly	Arg	Asp	Ser	Glu	
			515					520					525				
	Glu	Leu	Val	Ser	Gly	Leu	Ser	Ser	Val	Ser	Gly	Val	Glu	Val	Gly	Val	
		530					535					540					
45	Gly	Val	Gly	Ala	Gly	Gly	Gly	Val	Val	Leu	Val	Phe	Ala	Gly	Gln	Gly	
		545				550					555					560	
	Cys	Gln	Trp	Val	Gly	Met	Gly	Arg	Glu	Leu	Leu	Gly	Ser	Ser	Leu	Val	
				565						570					575		
50	Phe	Ala	Glu	Ser	Met	Arg	Glu	Cys	Ala	Ala	Ala	Leu	Ser	Pro	Phe	Val	
				580					585					590			
	Asp	Phe	Ser	Val	Val	Asp	Val	Leu	Gly	Ser	Ala	Gly	Glu	Leu	Gly	Arg	
			595					600					605				
55	Val	Glu	Val	Val	Gln	Pro	Ala	Leu	Trp	Ala	Val	Met	Val	Ser	Leu	Ala	

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	610					615						620					
	Arg	Val	Trp	Arg	Ser	Trp	Gly	Val	Pro	Val	Ala	Ala	Val	Val	Gly	His	
	625					630					635					640	
5		Ser	Gln	Gly	Glu	Ile	Ala	Ala	Ala	Thr	Val	Ala	Gly	Ala	Leu	Ser	Val
					645						650				655		
	Gly	Asp	Ala	Ala	Arg	Val	Val	Ala	Leu	Arg	Ser	Arg	Leu	Ile	Ala	Glu	
10				660					665					670			
	Arg	Leu	Ser	Gly	Leu	Gly	Gly	Met	Val	Ser	Val	Ala	Leu	Ser	Arg	Glu	
			675					680					685				
	Arg	Val	Val	Ser	Leu	Ile	Ala	Gly	Val	Pro	Gly	Val	Ser	Val	Ala	Ala	
15				690			695					700					
	Val	Asn	Gly	Ser	Ser	Ser	Thr	Val	Val	Ser	Gly	Glu	Ala	Ala	Gly	Leu	
	705					710					715					720	
	Glu	Arg	Val	Leu	Ala	Ala	Cys	Val	Ser	Ser	Gly	Val	Arg	Ala	Arg	Arg	
20					725					730					735		
	Ile	Asp	Val	Asp	Tyr	Ala	Ser	His	Ser	Val	Gln	Val	Glu	Leu	Ile	Arg	
				740					745					750			
	Glu	Glu	Leu	Leu	Gly	Val	Leu	Asp	Gly	Ile	Val	Pro	Arg	Ser	Gly	Glu	
25			755					760					765				
	Ile	Pro	Phe	Val	Ser	Thr	Val	Thr	Gly	Glu	Arg	Ile	Asp	Thr	Val	Glu	
		770					775					780					
	Leu	Gly	Ala	Glu	Tyr	Trp	Tyr	Arg	Asn	Leu	Arg	Gln	Thr	Val	Glu	Phe	
30						790					795					800	
	Gln	Ala	Ser	Val	Gln	Thr	Leu	Leu	Ala	Gln	Gly	His	Gln	Val	Phe	Leu	
					805					810					815		
	Glu	Ser	Ser	Pro	His	Pro	Val	Leu	Thr	Val	Gly	Ile	Glu	Glu	Thr	Val	
35				820					825					830			
	His	Glu	Ser	Ala	Ala	Gln	Ala	Val	Val	Leu	Gly	Ser	Leu	Arg	Arg	Asp	
			835					840					845				
	Glu	Gly	Ala	Leu	Thr	Arg	Leu	Val	Thr	Ser	Ala	Gly	Glu	Ala	Trp	Ala	
40			850				855					860					
	Arg	Gly	Val	Pro	Val	Asp	Trp	Ala	Gly	Met	Leu	Ala	Gly	Gly	Arg	Arg	
	865					870					875					880	
	Val	Glu	Leu	Pro	Thr	Tyr	Pro	Phe	Leu	Arg	Glu	Arg	Leu	Trp	Leu	Glu	
45					885					890					895		
	Pro	Ser	Arg	Ser	Arg	Thr	Gly	Asn	Leu	Asn	Met	Ala	Gly	Leu	Val	Glu	
				900					905					910			
	Ala	Gly	His	Glu	Ile	Leu	Pro	Ala	Ala	Val	Glu	Leu	Pro	Gly	Glu	Gln	
50				915				920					925				
	Trp	Val	Trp	Thr	Gly	Glu	Leu	Ser	Leu	Ser	Ala	Tyr	Pro	Trp	Leu	Ala	
							935					940					
	Asp	His	Gln	Val	Leu	Gly	Gln	Thr	Leu	Val	Pro	Gly	Val	Ala	Trp	Val	
55						950					955					960	

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	Glu Leu Ala Leu His Ala Gly His Gln Leu Gly Phe Gly Ser Val Glu	965	970	975
5	Glu Leu Thr Leu Gln Ala Pro Leu Val Leu Gly Glu Ser Asp Ala Val	980	985	990
	Gln Val Arg Val Val Val Ser Asp Leu Gly Glu Ser Asp Arg Arg Ala	995	1000	1005
10	Val Ser Val His Ser Arg Gly Asp Asp Gln Thr Trp Val Thr His Ala	1010	1015	1020
	Glu Gly Phe Leu Thr Ala Lys Gly Ala Gln Pro Glu Thr Met Ala Val	1025	1030	1035
15	Trp Pro Pro Ser Gly Ala Glu Pro Val Glu Ala Asp Gly Phe Tyr Glu	1045	1050	1055
	Arg Leu Ala Asp Ala Gly Tyr His Tyr Gly Pro Val Phe Gln Gly Val	1060	1065	1070
20	Ser Lys Val Trp Arg Ala Gly Glu Glu Ile Tyr Ala Glu Val Gly Leu	1075	1080	1085
	Leu Asp Asp Ala Asp Val Asp Gly Phe Gly Ile His Pro Ala Leu Leu	1090	1095	1100
25	Asp Ala Ala Leu Gln Thr Ala Tyr Val Ala Gln Arg Gly Pro Ala Glu	1105	1110	1115
	Thr Lys Leu Pro Phe Ala Phe Gly Asp Val Gln Leu Phe Ala Thr Gly	1125	1130	1135
30	Ala Arg Ser Leu Arg Val Arg Val Ser Pro Ala Ala Gln Gln Gly Met	1140	1145	1150
	Ala Trp Glu Ala Trp Asp Pro Thr Gly Leu Pro Val Phe Ser Leu Gly	1155	1160	1165
35	Tyr Leu Ala Thr Arg Pro Val Asp Arg Gly Gln Leu Thr Val Lys Arg	1170	1175	1180
	Pro Glu Ser Leu Phe Lys Val Ala Trp Asp Glu Thr Val Pro Val Val	1185	1190	1195
40	Gly Asn Ala Thr Ala Ala His Gly Val Val Leu Gly Asp Asp Pro Phe	1205	1210	1215
	Ala Leu Gly Ala Ala Leu Arg Ala Ala Gly Trp Glu Val Gly Ala Ala	1220	1225	1230
45	Pro Glu Pro Ala Ser Ala Asp Thr Ala Ala Glu Val Leu Leu Leu Pro	1235	1240	1245
	Cys Thr Ala Pro Gly Glu Pro Asp Ala Asp Leu Pro Thr Ala Val Arg	1250	1255	1260
50	Ala Val Thr Ala Arg Val Leu Gly Val Leu Gln Glu Trp Leu Ala Asp	1265	1270	1275
	Glu Arg Leu Ala Gly Thr Arg Leu Ala Val Val Thr Arg Asn Ala Leu	1285	1290	1295
55				

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	Pro Gly Asp Leu Leu His Ser Pro Val Trp Gly Leu Val Arg Ser Ala	
	1300	1305 1310
5	Gln Thr Glu Asn Pro Gly Arg Ile Thr Leu Val Asp Leu Asp Asp His	
	1315	1320 1325
	Pro Asp Ser Ala Ala Val Leu Ala Glu Ala Val Gln Ser Asp Glu Pro	
	1330	1335 1340
10	Arg Ile Met Val Arg Glu Gly Arg Pro Thr Ala Ala Arg Leu Val Arg	
	1345	1350 1355 1360
	Ala Thr Ala Pro Glu Leu Val Pro Pro Ala Gly Ala Asp Ala Trp Arg	
	1365	1370 1375
15	Leu Glu Ile Thr Glu Pro Gly Thr Phe Asp Asn Leu Thr Leu Gly Val	
	1380	1385 1390
	Tyr Pro His Ala Glu Lys Thr Leu Ala Asp Asn Glu Val Arg Val Ala	
	1395	1400 1405
20	Val His Ala Gly Gly Leu Asn Phe His Asp Val Val Ala Ala Leu Gly	
	1410	1415 1420
	Met Val Glu Asp Asp Leu Thr Leu Gly Arg Glu Ala Ala Gly Val Val	
	1425	1430 1435 1440
25	Val Glu Val Gly Asp Ala Val Pro Asp Leu Thr Pro Gly Asp His Val	
	1445	1450 1455
	Met Gly Ile Leu Ser Ser Gly Phe Gly Pro Leu Ala Val Thr Asp His	
	1460	1465 1470
30	Arg Tyr Leu Ala Arg Met Pro Glu Gly Trp Thr Phe Ala Gln Ala Ala	
	1475	1480 1485
	Ser Val Pro Ala Ala Phe Leu Thr Ala Tyr Tyr Gly Leu Cys Asp Leu	
	1490	1495 1500
35	Gly Gly Ile Arg Ala Gly Asp Arg Val Leu Ile His Ala Ala Ala Gly	
	1505	1510 1515 1520
	Gly Val Gly Met Ala Ala Val Gln Ile Ala Arg His Leu Gly Ala Glu	
	1525	1530 1535
40	Val Phe Gly Thr Ala Ser Pro Arg Lys Trp Gly Ala Leu Arg Ala Leu	
	1540	1545 1550
	Gly Leu Asp Asp Ala His Leu Ser Ser Arg Thr Leu Asp Phe Glu	
	1555	1560 1565
45	Gln Glu Phe Leu Asp Ala Thr Asp Gly Arg Gly Val Asp Leu Val Leu	
	1570	1575 1580
	Asn Ser Leu Ala Arg Glu Phe Val Asp Ala Ser Leu Arg Leu Met Pro	
	1585	1590 1595 1600
50	Gly Gly Gly Arg Phe Val Asp Met Gly Lys Thr Asp Ile Arg Arg Pro	
	1605	1610 1615
	Glu Gln Val Ala Glu Asp His Gly Gly Val Ala Tyr Gln Ala Phe Asp	
	1620	1625 1630
55	Leu Val Glu Ala Gly Pro Gln Arg Thr Gly Glu Met Leu Ala Glu Ile	

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	1635	1640	1645
5	Val Arg Leu Phe Gln Ala Gly Ala Phe Arg Pro Leu Pro Ile Thr Gln 1650 1655 1660		
	Trp Asp Val Arg Arg Ala Pro Glu Ala Phe Arg His Ile Ser Gln Ala 1665 1670 1675 1680		
10	Lys His Ile Gly Lys Ile Val Leu Thr Val Pro Arg Pro Ile Asp Thr 1685 1690 1695		
	Asp Gly Thr Val Met Val Thr Gly Ala Thr Gly Thr Leu Gly Gly Phe 1700 1705 1710		
15	Val Ala Arg His Leu Val Thr His His Gly Ile Arg Arg Leu Leu Leu 1715 1720 1725		
	Val Ser Arg Ser Ala Glu Arg Thr Asp Leu Val Arg Glu Leu Thr Glu 1730 1735 1740		
20	Leu Gly Ala Asp Val Thr Trp Ala Ser Cys Asp Leu Ala Asp Ala Thr 1745 1750 1755 1760		
	Ala Val Glu Glu Thr Val Arg Ser Val Asp Glu Arg His Pro Leu Val 1765 1770 1775		
25	Ala Val Val His Ser Ala Gly Val Leu Asp Asp Gly Val Ile Asp Lys 1780 1785 1790		
	Gln Ser Pro Glu Arg Leu Asp Thr Val Met Arg Pro Lys Val Asp Ala 1795 1800 1805		
30	Ala Trp Asn Leu His Arg Leu Leu Asp Asn Ala Pro Leu Ala Asp Phe 1810 1815 1820		
	Val Leu Phe Ser Ser Ala Ser Gly Val Leu Gly Gly Ala Gly Gln Ser 1825 1830 1835 1840		
35	Asn Tyr Ala Ala Ala Asn Ala Phe Leu Asp Ala Leu Ala Glu His Arg 1845 1850 1855		
	Arg Ala Gln Gly Leu Ala Gly Gln Ala Leu Ala Trp Gly Leu Trp Ser 1860 1865 1870		
40	Asp Arg Ser Thr Met Thr Gly Gln Leu Gly Ser Thr Glu Leu Ala Arg 1875 1880 1885		
	Ile Ala Arg Asn Gly Val Ala Glu Met Ser Glu Thr Glu Gly Leu Ala 1890 1895 1900		
45	Leu Phe Asp Ala Ala Arg Asp Thr Ala Glu Ala Val Leu Leu Pro Met 1905 1910 1915 1920		
	His Leu Asp Val Ala Arg Leu Arg Ser Arg Asn Gly Glu Val Pro Ala 1925 1930 1935		
50	Val Phe Arg Arg Leu Ile His Ala Thr Ala Arg Arg Thr Ala Ser Thr 1940 1945 1950		
	Ala Val Arg Ser Ala Gly Leu Glu Gln Gln Leu Ala Ser Leu Ser Gly 1955 1960 1965		
55	Pro Glu Arg Thr Glu Leu Leu Gly Leu Val Arg Asp His Ala Ala 1970 1975 1980		

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Ala Val Leu Gly His Gly Thr Ser Asp Ala Val Ser Pro Asp Arg Pro
1985 1990 1995 2000

5 Phe Arg Asp Leu Gly Phe Asp Ser Leu Thr Ala Val Glu Leu Arg Asn
2005 2010 2015

Arg Phe Ala Ala Leu Thr Gly Leu Arg Leu Pro Ala Thr Leu Val Phe
2020 2025 2030

10 Asp His Pro Ser Pro Thr Ala Leu Ala Gly His Leu Ala Gly Leu Leu
2035 2040 2045

Gly Ala Ala Thr Pro Ser Ala Ala Glu Pro Val Leu Ala Ala Val Gly
15 2050 2055 2060

Arg Leu Arg Ala Asp Leu Arg Ser Leu Thr Pro Asp Ala Glu Gly Ala
2065 2070 2075 2080

20 Glu Asp Val Thr Ile Gln Leu Glu Ala Leu Leu Ala Glu Trp Arg Glu
2085 2090 2095

Ala Ala Glu Lys Arg Ala Pro Glu Ala Val Gly Asp Glu Asp Leu Ser
2100 2105 2110

25 Thr Ala Thr Asp Asp Glu Ile Phe Ala Leu Val Asp Ser Glu Leu Gly
2115 2120 2125

Glu Ala
2130

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<210> 7
<211> 1742
<212> PRT
<213> Streptomyces parvulus Tü4055

35

<400> 7

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45

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	Met	Thr	Ala	Glu	Ala	Ser	Gln	Asp	Lys	Leu	Arg	Asp	Tyr	Leu	Arg	Lys
	1				5					10					15	
5	Thr	Leu	Ala	Asp	Leu	Arg	Thr	Thr	Lys	Gln	Arg	Leu	Arg	Asp	Thr	Glu
				20					25					30		
	Arg	Arg	Ala	Thr	Glu	Pro	Val	Ala	Ile	Val	Gly	Met	Ser	Cys	Arg	Leu
			35					40					45			
10	Pro	Gly	Asp	Val	Arg	Thr	Pro	Glu	Arg	Phe	Trp	Glu	Leu	Leu	Asp	Thr
		50					55					60				
	Gly	Thr	Asp	Ala	Leu	Thr	Pro	Leu	Pro	Thr	Asp	Arg	Gly	Trp	Asn	Leu
	65					70					75					80
15	Asp	Thr	Ala	Phe	Asp	Asp	Glu	Arg	Pro	Tyr	Arg	Arg	Glu	Gly	Gly	Phe
					85					90					95	
	Leu	Tyr	Asp	Ala	Gly	Arg	Phe	Asp	Ala	Glu	Phe	Phe	Gly	Ile	Ser	Pro
20				100					105					110		
	Arg	Glu	Ala	Leu	Ala	Met	Asp	Pro	Gln	Gln	Arg	Leu	Leu	Leu	Glu	Ser
			115					120					125			
25	Ser	Trp	Glu	Ala	Ile	Glu	His	Ala	Arg	Ile	Asp	Pro	Arg	Ser	Leu	His
	130						135					140				
30																
35																
40																
45																
50																
55																

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	Gly	Ser	Arg	Thr	Gly	Val	Trp	Phe	Gly	Thr	Ile	Gly	Gln	Asp	Tyr	Phe	145	150	155	160
5	Ser	Leu	Phe	Ala	Ala	Ser	Gly	Gly	Glu	His	Ala	Asn	Tyr	Leu	Ala	Thr	165	170	175	
	Ala	Cys	Ser	Ala	Ser	Val	Met	Ser	Gly	Arg	Val	Ser	Tyr	Val	Leu	Gly	180	185	190	
10	Leu	Glu	Gly	Pro	Ala	Val	Thr	Val	Asp	Thr	Ala	Cys	Ser	Ser	Ser	Leu	195	200	205	
	Val	Ala	Leu	His	Ser	Ala	Val	Gln	Ala	Leu	Arg	Ser	Gly	Glu	Cys	Glu	210	215	220	
15	Leu	Ala	Leu	Ala	Gly	Gly	Ala	Thr	Val	Met	Ala	Thr	Pro	Thr	Val	Phe	225	230	235	240
	Thr	Ala	Phe	Ser	His	Gln	Arg	Gly	Leu	Ala	Gly	Asp	Gly	Arg	Cys	Lys	245	250	255	
20	Ala	Phe	Ala	Ala	Gly	Ala	Asp	Gly	Ala	Gly	Phe	Ala	Glu	Gly	Val	Gly	260	265	270	
	Val	Leu	Val	Leu	Glu	Arg	Leu	Ser	Val	Ala	Arg	Arg	Asn	Gly	His	Arg	275	280	285	
25	Val	Leu	Ala	Val	Val	Arg	Gly	Ser	Ala	Val	Asn	Gln	Asp	Gly	Ala	Ser	290	295	300	
	Asn	Gly	Leu	Thr	Ala	Pro	Asn	Gly	Pro	Ser	Gln	Gln	Arg	Val	Ile	Arg	305	310	315	320
30	Ala	Ala	Leu	Ala	Asn	Ala	Arg	Leu	Ala	Pro	Glu	Asp	Val	Asp	Ala	Val	325	330	335	
	Glu	Gly	His	Gly	Thr	Gly	Thr	Ser	Leu	Gly	Asp	Pro	Ile	Glu	Ala	Gln	340	345	350	
35	Ala	Leu	Leu	Ala	Thr	Tyr	Gly	Arg	Gly	Arg	Asp	Ala	Glu	Arg	Pro	Leu	355	360	365	
	Trp	Leu	Gly	Ser	Val	Lys	Ser	Asn	Ile	Gly	His	Ala	Gln	Ala	Ala	Ala	370	375	380	
40	Gly	Val	Ala	Gly	Val	Ile	Lys	Met	Val	Leu	Ala	Met	Glu	Lys	Gly	Arg	385	390	395	400
	Leu	Pro	Arg	Thr	Leu	His	Val	Asp	Glu	Pro	Ser	Gly	Glu	Val	Asp	Trp	405	410	415	
45	Asp	Ser	Gly	Ala	Val	Arg	Leu	Leu	Thr	Glu	Ala	Arg	Asp	Trp	Pro	Ser	420	425	430	
	Gly	Glu	Gly	Arg	Val	Arg	Arg	Ala	Gly	Val	Ser	Ser	Phe	Gly	Ile	Ser	435	440	445	
50	Gly	Thr	Asn	Ala	His	Val	Ile	Ile	Glu	Glu	Pro	Gln	Glu	Glu	Glu	Ala	450	455	460	
	Ala	Pro	Asp	Ser	Ser	Ala	Ser	Gly	Ala	Val	Pro	Trp	Val	Leu	Ser	Ala	465	470	475	480
55	Arg	Ser	Ala	Glu	Ala	Leu	Gln	Ala	Leu	Ala	Ser	Gln	Leu	Ala	Asp	His				

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	Ser Val Thr Ala Val Pro Thr Leu Arg Pro Asp His Asp Glu Ser Arg	
	835	840 845
5	Thr Val Leu Ser Ala Ala Ala Ser Leu Tyr Val Gln Gly His Pro Val	
	850	855 860
	Asp Trp Ala Pro Leu Phe Pro Arg Ala Arg Thr Val Asp Leu Pro Thr	
	865	870 875 880
10	Tyr Pro Phe Gln His Gln His Tyr Trp Met Glu Ser Ala Ala Arg Pro	
		885 890 895
	Thr Val Glu Asp Thr Pro Arg Glu Pro Leu Asp Gly Trp Thr His Arg	
		900 905 910
15	Ile Asp Trp Val Pro Leu Val Asp Glu Glu Pro Ala Pro Val Leu Ala	
		915 920 925
	Gly Thr Trp Leu Leu Val Arg Pro Glu Glu Gly Pro Arg Pro Leu Ala	
		930 935 940
20	Asp Ala Val Ala Asp Ala Leu Thr Arg His Gly Ala Ser Val Val Glu	
		945 950 955 960
	Ala Ala Arg Val Pro His Gln Ser Asp Thr Glu Leu Thr Gly Val Val	
		965 970 975
25	Ser Leu Leu Gly Pro Gly Ala Asp Gly Asp Gly Gly Leu Asp Ala Thr	
		980 985 990
	Leu Arg Leu Val Gln Asp Leu Ala Thr Ala Gly Ser Thr Ala Pro Leu	
		995 1000 1005
30	Trp Ile Val Thr Ser Gly Ala Val Ala Val Gly Thr Ser Asp Thr Val	
		1010 1015 1020
	Pro Asn Pro Glu Gln Ala Thr Leu Trp Gly Leu Ala Arg Ala Ala Ala	
		1025 1030 1035 1040
35	Thr Glu Trp Pro Gly Leu Gly Ala Ala Arg Ile Asp Leu Pro Ala Asp	
		1045 1050 1055
	Leu Thr Glu Gln Val Gly Arg Arg Leu Cys Ala Arg Leu Leu Asp Arg	
		1060 1065 1070
40	Ser Glu Gln Glu Thr Ala Val Arg Gln Ala Gly Val Phe Ala Arg Arg	
		1075 1080 1085
	Leu Val Arg Ala Arg Thr Ser Asp Gly Arg Trp Thr Pro Arg Gly Thr	
		1090 1095 1100
45	Val Leu Val Thr Gly Gly Thr Gly Ala Leu Ala Gly His Val Ala Arg	
		1105 1110 1115 1120
	Trp Leu Ala Glu Glu Gly Ala Glu His Ile Val Leu Ala Gly Arg Arg	
		1125 1130 1135
50	Gly Pro Asp Gly Gln Gly Ala Glu Ala Leu Arg Ala Asp Leu Val Ala	
		1140 1145 1150
	Ala Gly Val Lys Ala Thr Ile Val Arg Cys Asp Val Ala Asp Arg Asp	
		1155 1160 1165
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	1170	1175	1180
5	Thr Ala Gly Val Val Asp Asp	Gly Leu Leu Thr Ser	Leu Thr Pro Ala
	1185	1190	1195 1200
	Gln Val Glu Arg Val Leu Arg	Pro Lys Leu Leu Gly	Ala Arg Asn Leu
		1205	1210 1215
10	His Glu Leu Thr Arg Asp Arg	Glu Leu Asp Ala Phe	Val Leu Phe Ser
		1220 1225	1230
	Ser Leu Ala Gly Val Leu Gly	Gly Ala Gly Gln Ala Asn	Tyr Ala Ala
		1235	1240 1245
15	Ala Asn Ala Tyr Leu Asp Ala	Leu Ala Ala His Arg	Thr Ala His Gly
		1250 1255	1260
	Leu Pro Ala Ala Ser Leu Ala	Trp Gly Pro Trp Glu Gly	Asp Gly Met
		1265 1270	1275 1280
20	Ala Ala Ala Gln Glu Ala Ala	Asp Arg Leu Arg Arg	Ser Gly Leu Thr
		1285	1290 1295
	Pro Leu Pro Pro Glu Gln Ala	Val Arg Ala Leu Gly	Arg Gly His Gly
		1300	1305 1310
25	Pro Leu Val Val Ala Asp Ala	Asp Trp Ala Arg Leu Ala	Ala Gly Ser
		1315 1320	1325
	Thr Gln Arg Leu Leu Asp Glu	Leu Pro Glu Val Arg	Ala Val Arg Pro
		1330 1335	1340
30	Ala Glu Pro Ala Val Gly Gln	Arg Pro Asp Leu Pro Ala	Arg Leu Ala
		1345 1350	1355 1360
	Gly Arg Pro Ala Glu Glu Gln	Ser Ala Val Leu Leu Glu	Ala Val Arg
		1365	1370 1375
35	Glu Glu Ile Ala Ala Val Leu	Arg Tyr Ala Asp Pro Ala	Arg Ile Gly
		1380 1385	1390
	Ala Asp His Glu Phe Leu Ala	Leu Gly Phe Asp Ser Leu	Thr Ser Ile
		1395	1400 1405
40	Glu Leu Arg Asn Arg Leu Ala	Thr Arg Ile Gly Leu Thr	Leu Pro Ala
		1410 1415	1420
	Thr Leu Thr Leu Glu Gln Arg	Thr Pro Ala Gly Leu Ala	Ala His Leu
		1425 1430	1435 1440
45	Arg Glu Arg Ile Ala Asp Arg	Pro Val Gly Ser Gly Ala	Val Pro Val
		1445	1450 1455
	Pro Gly Ser Ala Asp Val Pro	Glu Ala Gly Gly Gly Ser	Gly Leu Gly
		1460	1465 1470
50	Glu Leu Trp Gln Glu Ala Asp	Arg His Gly Arg Arg Leu	Glu Phe Ile
		1475	1480 1485
	Asp Val Leu Thr Ala Ala Ala	Ala Phe Arg Pro Ala Tyr	Arg Glu Pro
		1490	1495 1500
55	Ala Glu Leu Glu Leu Pro Pro	Leu Arg Leu Thr Ser Gly	Gly Asp Glu

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	1505		1510		1515		1520									
	Pro	Pro	Leu	Phe	Cys	Ile	Pro	Ser	His	Leu	Gly	Lys	Ala	Asp	Pro	His
					1525					1530					1535	
5	Lys	Phe	Leu	Arg	Phe	Ala	Ala	Ala	Leu	Arg	Gly	Arg	Arg	Asp	Val	Phe
				1540					1545					1550		
	Val	Leu	Arg	Gln	Pro	Gly	Phe	Val	Pro	Gly	Gln	Pro	Leu	Pro	Ala	Gly
10			1555				1560					1565				
	Leu	Asp	Val	Leu	Leu	Asp	Thr	His	Ala	Arg	Ala	Met	Ala	Gly	His	Asp
		1570					1575					1580				
15	Arg	Pro	Val	Leu	Leu	Gly	Tyr	Ser	Ala	Gly	Gly	Leu	Ala	Ala	Gln	Ala
	1585					1590					1595					1600
	Leu	Ala	Ala	Arg	Leu	Ala	Glu	Leu	Gly	Arg	Pro	Pro	Ala	Ala	Val	Val
				1605					1610						1615	
20	Leu	Val	Asp	Thr	Tyr	Ala	Pro	Asp	Glu	Thr	Glu	Val	Met	Ala	Arg	Ile
			1620						1625					1630		
	Gln	Gly	Ala	Met	Glu	Gln	Gly	Gln	Arg	Asp	Arg	Asp	Gly	Arg	Thr	Gly
25			1635					1640					1645			
	Ala	Ala	Phe	Gly	Glu	Ala	Trp	Leu	Thr	Ala	Met	Gly	His	Tyr	Phe	Gly
		1650					1655					1660				
30	Phe	Asp	Trp	Thr	Pro	Cys	Pro	Val	Asp	Val	Pro	Val	Leu	His	Val	Arg
	1665					1670				1675						1680
	Ala	Gly	Asp	Pro	Met	Thr	Gly	Met	Pro	Val	Glu	Gly	Arg	Trp	Gln	Ala
				1685					1690						1695	
35	Arg	Trp	Asn	Leu	Pro	His	Thr	Ala	Val	Asp	Val	Pro	Gly	Asp	His	Phe
			1700						1705					1710		
	Thr	Met	Met	Glu	Asp	His	Ala	Pro	Arg	Thr	Ala	Asp	Thr	Val	His	Asp
		1715					1720					1725				
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<213> Streptomyces parvulus Tü4055

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5	Pro	Ala	His	Pro	Thr	Ala	Pro	Gln	Leu	Ile	Cys	Leu	Pro	His	Ala	Gly	
				20					25					30			
	Gly	Ser	Ala	Thr	Phe	Tyr	His	Pro	Val	Ala	Ala	Ala	Leu	Ala	Pro	Arg	
			35					40					45				
10	Cys	Asp	Val	Leu	Ala	Val	Gln	Tyr	Pro	Gly	Arg	Gln	Asp	Arg	Arg	Ala	
		50					55					60					
	Glu	Lys	Pro	Leu	Glu	Asp	Ile	Asp	Glu	Leu	Ala	Asn	Gln	Leu	Phe	Pro	
15		65				70					75					80	
	Val	Leu	Arg	Ala	Arg	Val	His	Gln	Pro	Val	Ala	Leu	Phe	Gly	His	Ser	
					85					90					95		
20	Met	Gly	Ala	Thr	Leu	Ala	Phe	Glu	Leu	Ala	Arg	Arg	Phe	Glu	Ser	Ala	
				100					105					110			
	Gly	Ile	Ser	Leu	Glu	Ala	Leu	Leu	Val	Ser	Ala	Arg	Pro	Ala	Pro	Ser	
25			115					120					125				
	Arg	Gln	Arg	Thr	Gly	Gly	Thr	Val	His	Leu	Leu	Ser	Asp	Glu	Glu	Leu	
		130					135					140					
30	Val	Ala	Glu	Leu	Arg	Thr	Leu	Asp	Gly	Thr	Ala	Glu	Gln	Val	Phe	His	
		145				150				155						160	
	Asp	Glu	Glu	Leu	Val	Arg	Met	Ala	Leu	Pro	Ala	Ile	Arg	Gly	Asp	Tyr	
				165					170						175		
35	Arg	Ala	Ala	Glu	Thr	Tyr	Arg	Tyr	Arg	Pro	Gly	Pro	Lys	Leu	Arg	Cys	
			180						185					190			
	Pro	Ile	His	Ala	Leu	Thr	Gly	Asp	Asp	Asp	Pro	Met	Val	Thr	Pro	Val	
40			195					200					205				
	Glu	Ala	Arg	Ala	Trp	Ser	Glu	His	Thr	Asp	Gly	Pro	Phe	Thr	Leu	Asp	
		210					215					220					
45	Thr	Phe	Ala	Gly	Gly	His	Phe	Tyr	Leu	Leu	Glu	His	Arg	Asp	Ala	Ile	
		225				230					235					240	
	Leu	Gly	Ile	Ile	Ala	Glu	His	Leu	Arg	Thr	Cys	Ser	Arg	Ala	Pro	Gly	
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<213> Streptomyces parvulus Tü4055

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5	Ser	Gly	Ile	Gly	Arg	Ala	Ala	Ala	Arg	Leu	Leu	Val	Gly	His	Gly	Ala	
				20					25					30			
	Arg	Val	Val	Leu	Val	Gly	Arg	Thr	Glu	Ser	Ala	Leu	Thr	Glu	Thr	Thr	
			35					40					45				
10	Ala	Gly	Leu	Pro	Ser	Ser	His	His	Leu	Val	Val	Pro	Cys	Asp	Val	Gly	
		50					55					60					
	Asp	Asp	Lys	Gln	Val	Ala	Asp	Cys	Val	Ala	Arg	Ala	Val	Ser	Arg	Phe	
15		65				70					75					80	
	Gly	Arg	Leu	Asp	Gly	Ala	Phe	Asn	Asn	Ala	Gly	Thr	Phe	Gly	Ser	Phe	
				85					90						95		
	Gly	Pro	Leu	His	Gln	Asp	Thr	Ala	Asp	Asn	Phe	Asp	Arg	Val	Ile	Ala	
20				100					105					110			
	Thr	Asn	Leu	Arg	Gly	Val	Trp	Ser	Cys	Met	Arg	Gly	Gln	Ile	Glu	Ala	
25			115					120					125				
	Met	Leu	Thr	Ala	Gly	Gly	Gly	Ala	Ile	Val	Asn	Cys	Ala	Ser	Val	Ala	
		130					135					140					
	Gly	His	Ile	Gly	His	Ala	Gln	Ser	Pro	Leu	Tyr	Ser	Ala	Thr	Lys	His	
30		145				150					155					160	
	Ala	Val	Ile	Gly	Leu	Ser	Lys	Ser	Val	Ala	Leu	Gln	Tyr	Ala	Gly	Asp	
				165					170						175		
	Gly	Ile	Arg	Val	Asn	Val	Val	Ser	Pro	Gly	Ser	Thr	Asp	Thr	Pro	Met	
35				180					185					190			
	Leu	Arg	Ser	Leu	Tyr	Ala	Asp	Pro	Ser	Ala	Leu	Ala	Gln	Arg	Ala	Arg	
			195					200					205				
40	Arg	Ala	Pro	Leu	Gly	Arg	Leu	Gly	Lys	Cys	Glu	Glu	Val	Ala	Asn	Ala	
		210					215					220					
	Val	Val	Trp	Leu	Leu	Ser	Pro	Leu	Ala	Ala	Tyr	Val	Thr	Gly	Gln	Thr	
45		225				230					235					240	
	Leu	Gly	Val	Asp	Gly	Gly	Val	Thr	Ala	Gly	Ser	Ala	Ile	Pro	Arg	Thr	
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50	Asn	Ala	Thr	Pro	Glu	Gly	Gln	His	Arg								
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<213> Streptomyces parvulus Tü4055

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5	Ile	Cys	Ala	Glu	Val	Ala	Arg	Gly	Leu	Ala	Ala	Arg	Gly	Leu	Arg	Val	
				20					25					30			
	Val	Leu	Leu	Asp	Lys	Asp	Ala	Glu	Ala	Val	His	Arg	Val	Ala	Asp	Gly	
			35					40					45				
10	Leu	Gly	Asp	Arg	Leu	Ala	Arg	Asp	Pro	Leu	Val	Ala	Asp	Val	Thr	Asp	
		50					55					60					
	Pro	His	Ala	Leu	Ala	Ser	Ala	Val	Asp	Ser	Leu	Ala	Pro	Gln	His	Arg	
	65					70					75					80	
15	Pro	Gly	Val	Leu	Val	Asn	Gly	Val	Gly	Gly	Asp	Thr	Arg	Ala	Arg	Ser	
				85					90						95		
	Val	Thr	Glu	Leu	Thr	Glu	Ala	Asp	Leu	Gln	Glu	Ala	Val	Thr	His	Asn	
20				100					105					110			
	Leu	Ala	Ser	Val	Phe	Thr	Met	Thr	Arg	Leu	Cys	Val	Pro	Ala	Met	Val	
			115					120					125				
25	Ala	Ala	Gly	Trp	Gly	Arg	Val	Val	Asn	Leu	Ala	Ser	Val	Ala	Gly	Arg	
		130					135					140					
	Thr	Tyr	Thr	Arg	Phe	Ser	Asn	Ala	Ala	Tyr	Val	Ala	Ala	Lys	Ala	Gly	
30																	
	145					150					155					160	
	Val	Ile	Gly	Phe	Thr	Lys	Gln	Cys	Ala	Tyr	Glu	Leu	Ala	Pro	His	Gly	
				165						170					175		
35	Val	Thr	Val	Asn	Ala	Val	Ala	His	Gly	Val	Ile	Gly	Thr	Glu	Arg	Ile	
				180					185					190			
	Arg	Arg	Ala	Trp	Glu	Asp	Lys	Pro	Pro	Gln	Trp	Thr	Ala	Asp	Arg	Val	
40			195					200					205				
	Ser	His	Ile	Pro	Ala	Gly	Arg	Phe	Gly	Ser	Val	Ala	Glu	Ala	Ala	Gly	
		210					215					220					
45	Met	Val	Cys	His	Leu	Cys	Gly	Glu	Asp	Ala	Gly	Tyr	Thr	Thr	Gly	Thr	
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	Val	Val	Asp	Val	Asn	Gly	Gly	Leu	His	Ile							
				245						250							

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	Met	Ile	Arg	Arg	Val	Arg	Leu	His	Thr	Ala	Val	Val	Pro	Met	Ala	Ala
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5	Ala	Phe	Asp	His	Ala	Thr	Arg	Ser	Arg	Arg	Ser	Ala	Ala	Ser	Leu	Leu
				20					25					30		
	Val	Glu	Ile	Glu	Leu	Ala	Gly	Thr	Arg	Gly	Trp	Gly	Glu	Gly	Ala	Pro
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	Gln	Ala	Cys	Asp	Pro	Gly	Glu	Leu	Ala	Glu	Arg	Ile	Glu	Trp	Arg	Asp
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15	Phe	Glu	Ser	Ala	Val	Ala	Ser	Ile	Ala	Gln	Leu	Pro	Leu	Thr	Gly	Leu
					85					90					95	
	Val	Asp	Gly	Ser	Ser	Ala	Ala	Ala	Ala	Val	Glu	Ile	Ala	Leu	Leu	Asp
20				100					105					110		
	Ala	Val	Cys	Arg	His	Phe	Ala	Arg	Pro	Leu	Ala	Asp	Val	Leu	Arg	Val
			115					120					125			
25	Leu	Ala	Pro	Pro	Ala	Arg	Ser	Arg	Arg	Asp	Gly	Pro	Thr	Ser	Val	Ser
		130					135					140				
	Leu	Val	Ile	His	Leu	Ser	Arg	Asp	Val	Ala	Thr	Val	Leu	Asp	Ala	Leu
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30	Thr	Pro	Arg	Ala	Leu	Ala	Ala	Leu	Arg	His	Val	Lys	Ile	Lys	Val	Ala
				165					170						175	
	Asp	Pro	Ala	Gly	Ala	Val	Asp	Arg	Leu	Thr	Ala	Ala	Gln	Asp	Arg	Leu
				180					185					192		
35	Pro	Ala	Asp	Thr	Arg	Val	Ser	Leu	Asp	Val	Asn	Gly	Ala	Trp	Thr	Ala
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	Glu	Glu	Ala	Glu	Lys	Val	Ala	Gly	Glu	Leu	Asp	Gly	Val	Gly	Trp	Val	
	210						215					220					
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	225					230					235					240	
	Arg	Ala	Thr	Gly	Leu	Pro	Val	Met	Leu	Asp	Glu	Ser	Cys	Thr	Gly	Pro	
					245					250						255	
10	Ala	Asp	Leu	His	Ala	Ala	Ala	Thr	Ser	Gly	Ala	Ala	Ser	His	Ile	Asn	
				260					265						270		
	Val	Arg	Leu	Ser	Lys	Cys	Gly	Gly	Phe	Leu	Ala	Ala	Ala	Arg	Leu	Ala	
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	Leu	Arg	Ala	Asp	Glu	Leu	Gly	Val	Gly	Cys	Gln	Leu	Gly	Val	His	Val	
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	Ala	Glu	Val	Gly	Pro	Leu	Trp	Ala	Ala	Gly	Arg	Thr	Leu	Ala	Thr	Ala	
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	Trp	Asp	Leu	Trp	Gln	Thr	Val	Glu	Ala	Gly	Arg	Ala	Asp	Glu	Trp	Phe	
					325					330						335	
25	Pro	Val	Pro	Leu	Thr	Thr	Pro	Ala	Phe	Thr	Val	Asp	Arg	Ser	Leu	His	
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30	Glu	Leu	Leu	Arg	His	Thr	Arg	Cys	Ala	Ala	Thr	Trp	Glu	Ser	Gly	Gly	
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 His Gly Ile Gln Ser His Ala Gly Trp Leu Phe Glu Thr Gly Pro Glu
 35 40 45
 10 Leu Asn Ala Arg Gly Ile Asp Val Tyr Ala Leu Asp Arg Arg Gly Ser
 50 55 60
 Gly Arg Ser Glu Gly Pro Arg Gly His Leu Pro Ser Ala Asp Leu Val
 65 70 75 80
 15 Leu Asp Asp Tyr Ala Arg Ala Leu Asp Ala Val Thr Ala Glu Val Gly
 85 90 95
 20 Gly Ala Gly Pro Val Ala Leu Gly Gln Ser Leu Gly Gly Ser Val Leu
 100 105 110
 25 Ala Ala Leu Trp Cys Thr Arg Asp Leu Pro Val Arg Arg Leu Val Leu
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 Cys Ala Pro Ala Leu Gly Gln Gln Arg Ala Arg His Thr Ala Asp Thr
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 30 Leu Ala Glu Arg Arg Ala Leu Thr Gly Ser Gly Leu Arg Pro Val Gly
 145 150 155 160
 Leu Ala Asp Gly Asp Tyr Thr Asp Leu Pro Arg Tyr Arg Glu Phe Leu
 165 170 175
 35 Thr Gly Asp His Leu Met Leu Arg Glu Val Thr Ser Ala Thr Gln Ala
 180 185 190
 Thr Leu Val His Leu Glu Asp His Tyr Ala Arg Gly Ala Pro Arg Thr
 195 200 205
 40 Arg Leu Pro Val Asp Leu Ala Leu Pro Thr His Asp Pro Ile Ile Asp
 210 215 220
 45 Leu Ser Ala Ala Arg Ala Met Leu Arg Arg Leu Thr Ser Ala Val His
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<213> Streptomyces parvulus Tü4055

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5	Asp	Thr	Val	Phe	Gly	Leu	Met	Gly	Asn	Ala	Asn	Leu	Leu	Tyr	Leu	Pro	
				20					25					30			
	Ala	Phe	Ala	Asp	Ala	Gly	Gly	Arg	Phe	Val	Ala	Val	Ala	His	Glu	Ala	
			35					40					45				
10	Gly	Ala	Val	Ala	Met	Ala	Asp	Gly	Arg	Ala	Arg	Met	Cys	Gly	Gly	Ile	
	50						55					60					
	Gly	Val	Ala	Ser	Val	Thr	His	Gly	Pro	Ala	Phe	Thr	Asn	Ala	Leu	Thr	
	65					70					75					80	
15	Pro	Leu	Val	Glu	Ala	Ala	Arg	Ser	His	Ser	Gln	Val	Leu	Leu	Ile	Thr	
					85					90					95		
	Gly	Asp	Pro	Pro	Pro	Val	Pro	Thr	His	Phe	His	His	Phe	Asp	Ile	Ala	
20				100					105					110			
	Thr	Val	Ala	Ala	Ala	Ala	Gly	Ala	Gly	Tyr	Glu	Arg	Val	His	Arg	Pro	
			115					120					125				
25	Ala	Ser	Leu	Val	Ala	Asp	Leu	Asn	Arg	Ala	Val	Gln	Arg	Ile	Val	Ala	
	130						135					140					
	Glu	Arg	Arg	Pro	Val	Val	Leu	Asn	Val	Pro	Ile	Asp	Leu	Met	Gln	Ala	
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40																	
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	145					150						155				160
	Glu	Ala	Gly	Glu	Gln	Ala	Pro	Val	Thr	Leu	Pro	Val	Ala	Pro	Gly	Pro
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	Gly	Ser	Ala	Lys	Arg	Pro	Leu	Val	Leu	Ala	Gly	His	Gly	Ala	Ala	Val
10			195					200					205			
	Ala	Gly	Ala	Arg	Glu	Ala	Leu	Val	Glu	Leu	Ala	Asp	Arg	Thr	Gly	Ala
		210					215					220				
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	Arg	Asp	Val	Gly	Ile	Phe	Gly	Ser	Leu	Ala	His	Ser	Val	Ala	Ser	Thr
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	Met	Trp	Thr	Val	Leu	Asn	Gly	Glu	Leu	Leu	Arg	Gly	Lys	Arg	Val	Val
			275					280					285			
	His	Val	Asp	Thr	Asp	Pro	Ala	Arg	Phe	Gly	Ser	Tyr	Ser	Pro	Val	Asp
25		290					295					300				
	Glu	Pro	Val	Ala	Gly	Asp	Ala	Arg	Arg	Thr	Ala	Glu	Thr	Met	Asn	Val
		305				310					315					320
	Leu	Leu	Asp	Gln	Ala	Gly	Val	Thr	Ala	Ala	Asn	Gly	Ala	Trp	Ala	Glu
30					325					330					335	
	Arg	Val	Ala	Gly	Gln	Leu	Ala	Gly	Phe	Ser	Pro	Gln	Asp	Asp	Val	Asp
				340					345					350		
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35			355					360					365			
	Leu	Asp	Arg	Ile	Leu	Pro	Ala	Glu	Arg	Ser	Val	Val	Ser	Asp	Ile	Gly
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	Arg	Phe	Asp	Val	Gly	Val	Trp	Pro	Tyr	Leu	Arg	Val	Ala	Asp	Pro	Leu
40		385				390					395					400
	His	Phe	Thr	Val	Met	Gly	Gly	Phe	Gly	Ser	Ile	Gly	Leu	Gly	Val	Ala
					405					410					415	
	Gly	Ala	Ile	Gly	Ala	Ala	Thr	Ala	Gly	Thr	Gly	Arg	Pro	Val	Val	Ala
45				420					425					430		
	Ala	Val	Gly	Asp	Gly	Gly	Phe	Met	Met	His	Leu	Ser	Glu	Phe	Thr	Thr
			435					440					445			
	Ala	Val	Arg	Tyr	Arg	Leu	Pro	Leu	Val	Val	Val	Val	Leu	Asn	Asp	Gly
50			450				455					460				
	Ala	Tyr	Gly	Ala	Glu	His	Tyr	Lys	Leu	Arg	Asn	His	Gly	Tyr	Asp	Pro
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	Ala	Tyr	Ser	Ala	Phe	Ala	Trp	Pro	Asp	Leu	Ala	Gly	Leu	Ala	Thr	Ala
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Met Gly Ala Arg Ala Leu Thr Val Arg Lys Ala Glu Glu Leu Asp Ala
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5 Val Gly Asp Leu Leu Ser Thr Leu Glu Gly Pro Leu Leu Val Asp Val
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5	Gly	Ile	Ala	Val	Leu	Met	Ala	Gly	Arg	Ser	Thr	Pro	Ala	Ala	Leu	Leu	
				20				25					30				
	Gly	Arg	Leu	Leu	Ala	Asp	Leu	Gly	Ala	Arg	Val	Val	Thr	Leu	Cys	Arg	
			35					40					45				
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		50					55					60					
	Gln	Ser	Ala	Ser	Gly	Trp	Asp	Gln	Ala	Ser	Arg	Leu	Leu	Gln	Thr	Ala	
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					85					90					95		
	Gly	Leu	Gly	Ala	Pro	Glu	Leu	Pro	His	Arg	Ser	Pro	Glu	Leu	Val	Ala	
20				100					105					110			
	Val	Arg	Leu	Ser	Ala	Phe	Gly	Leu	Thr	Gly	Pro	Leu	Arg	Asp	Ala	Pro	
			115					120					125				
25	Ala	Thr	Glu	Arg	Thr	Leu	Gln	Ala	Leu	Ala	Gly	Leu	Thr	Ser	Ala	Thr	
		130					135					140					
	Gly	Thr	Glu	Gly	Glu	Pro	Ser	Val	Leu	Ser	Val	Val	Gly	Leu	Ala	Ser	
	145					150				155						160	
30	Arg	Thr	Ala	Ala	Leu	Ser	Gly	Leu	Ile	Ala	Val	Val	Ala	Gly	Leu	Ile	
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	Gly	Arg	Glu	Arg	Gly	Gly	Gly	Gly	Asp	Tyr	Leu	Asp	Ile	Ala	Glu	Phe	
				180					185					190			
35	Asp	Ser	Leu	Phe	Thr	Leu	Thr	Gly	Thr	Leu	Leu	Pro	Ser	Val	Ala	Leu	
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	Ala	Gly	Arg	Pro	Pro	Arg	Arg	Thr	Gly	Asn	Arg	His	Gly	Met	Ala	Ala	
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	Pro	Trp	Asn	Ser	Tyr	Thr	Cys	Gln	Asp	Ala	Pro	Val	Val	Ile	Cys	Thr	
	225					230					235					240	
45	Met	Gly	Glu	Pro	Ile	Trp	His	Arg	Leu	Thr	Ala	Val	Leu	Gly	Arg	Arg	
					245					250					255		

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5	Asn	Ala	Asp	Glu	Leu	Asp	Glu	Ile	Leu	Gly	Lys	Trp	Thr	Ala	Gly	Gln	
			275					280					285				
	Arg	Ala	Val	Asp	Val	Val	Thr	Ala	Leu	Arg	Ala	Ala	Gly	Ile	Pro	Cys	
		290					295					300					
10	Ala	Gln	Val	Ala	Ala	Pro	Glu	Glu	Val	Arg	Asp	Gly	Ala	Ala	Ala	Arg	
	305					310					315					320	
	Arg	Arg	Gly	Leu	Val	Thr	Asp	Pro	Ser	Gly	Thr	Pro	Gly	Ser	Pro	Leu	
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15	Arg	Ser	Leu	Ile	Pro	Ala	Val	Thr	Asp	Gly	Pro	Met	Pro	Arg	Gln	Gly	
				340					345					350			
	Gly	Leu	Trp	Glu	Pro	Ile	Ala	Arg	Gly	Thr	Pro	Pro	Leu	Arg	Gly	Val	
			355					360					365				
20	Arg	Leu	Leu	Glu	Val	Gly	Ser	Tyr	Thr	Ala	Gly	Pro	His	Ala	Gly	Arg	
		370					375					380					
	Leu	Leu	Ala	Gln	Leu	Gly	Ala	Asp	Val	Leu	Lys	Val	Glu	Pro	Pro	His	
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25	Gly	Glu	Gly	Ser	Arg	Arg	Leu	Ala	Gln	Gln	Val	Ala	Gly	Val	Gly	Tyr	
					405					410					415		
	Leu	Tyr	Tyr	Val	Asn	Asn	Ala	Gly	Lys	Arg	Ser	Cys	Arg	Leu	Asp	Leu	
				420					425					430			
30	Ala	Asp	Ala	Glu	Asp	Arg	Ala	Gly	Phe	Glu	Arg	Leu	Leu	Ala	Gly	Cys	
			435					440					445				
	Asp	Ile	Val	Leu	Thr	Asn	Leu	Ala	Ala	Asp	Thr	Leu	Thr	Ala	Gln	Gly	
		450					455					460					
35	Leu	Ala	Pro	Asp	Gln	Ile	Leu	Ser	Arg	His	Gly	Val	Val	His	Cys	Thr	
	465				470						475					480	
	Val	Thr	Gly	His	Gly	Leu	Ala	Ala	Ala	Asp	Arg	Ser	Val	Asp	Thr	Val	
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40	Ile	Gln	Ala	Glu	Ser	Gly	Ile	Met	Arg	Leu	Val	Gly	Gly	Pro	Gly	Ala	
				500					505					510			
	Gly	Leu	Arg	Thr	Pro	Val	Ser	Ser	Ala	Asp	Val	Leu	Gly	Ala	Tyr	Leu	
			515				520						525				
45	Ala	Ala	Ala	Ala	Ala	Val	Val	Ser	Thr	Tyr	Val	Arg	Leu	Arg	Thr	Glu	
		530					535					540					
	His	Gly	Cys	Ala	Ala	Asp	Val	Ala	Leu	Phe	Asp	Ser	Ala	Val	Trp	Leu	
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					565					570					575		
	Val	Arg	Ala	Ala	Asp	Gly	Thr	Val	Leu	Val	Asp	Ala	Glu	Gly	Pro	Pro	
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	595	600	605
5	Val Gly Val Pro Ala Ala Pro Leu His Asp Leu Thr Arg Ala Val Arg 610 615 620		
	His Pro Gln Val Leu Ala Arg Arg Met Ala Val Ala Arg Asp Cys Ala 625 630 635 640		
10	Gly Thr Thr Val Leu Ile Thr Gly Asn His Leu Arg Ser Leu Leu Arg 645 650 655		
	Glu Asp Pro Pro Pro Thr Cys Ala Pro Val Asp Gln Asn Asp Pro Val 660 665 670		
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	Ser	Phe	Pro	Val	Arg	Arg	Ser	Val	Pro	Asp	Thr	Pro	Pro	Ala	Glu	His	
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10	Leu	Glu	Leu	Leu	Lys	Glu	Ser	Gly	Gly	Val	Cys	Pro	Phe	Thr	Met	Glu	
	50						55					60					
	Asp	Gly	Arg	Pro	Ala	Trp	Leu	Ala	Ala	Ser	His	Asp	Ala	Val	Arg	Ser	
15	65					70					75					80	
	Leu	Leu	Ala	Asp	Arg	Arg	Ile	Ser	Asn	Asn	Pro	Ala	Lys	Thr	Pro	Pro	
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	Phe	Ser	Gln	Arg	Glu	Ala	Leu	Gln	Lys	Glu	Arg	Gly	Gln	Phe	Ser	Arg	
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	His	Leu	Phe	Asn	Met	Asp	Ser	Pro	Glu	His	Asp	Val	Ala	Arg	Arg	Met	
			115					120					125				
25	Ile	Ala	Glu	Asp	Phe	Thr	Pro	Arg	His	Ala	Glu	Ala	Val	Arg	Pro	Tyr	
	130						135					140					
	Phe	Glu	Glu	Val	Phe	Gly	Glu	Ile	Val	Asp	Glu	Val	Val	His	Lys	Gly	
	145					150				155						160	
30	Pro	Pro	Ala	Glu	Met	Ile	Glu	Ser	Phe	Ala	Phe	Pro	Val	Ala	Thr	Arg	
					165				170						175		
	Thr	Ile	Cys	Lys	Val	Leu	Asp	Ile	Pro	Glu	Asp	Asp	Cys	Glu	Tyr	Phe	
35				180					185					190			
	Gln	Lys	Arg	Thr	Glu	Gln	Ile	Ile	Glu	Met	Asp	Arg	Gly	Glu	Glu	Asn	
			195					200					205				
40	Leu	Glu	Ala	Val	Val	Glu	Leu	Arg	Arg	Tyr	Val	Asp	Ser	Val	Met	Gln	
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	225						230					235					240
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					245					250					255		
	Asp	Asn	Ala	Met	Phe	Leu	Leu	Val	Ala	Gly	His	Glu	Pro	Ser	Ala	Asn	
				260					265					270			
10	Met	Leu	Gly	Leu	Gly	Val	Leu	Ala	Leu	Ala	Glu	Phe	Pro	Asp	Val	Ala	
			275					280						285			
	Glu	Glu	Leu	Arg	Ala	Glu	Pro	His	Leu	Trp	Pro	Gly	Ala	Ile	Asp	Glu	
15			290				295					300					
	Met	Leu	Arg	Tyr	Tyr	Thr	Ile	Ala	Arg	Ala	Thr	Lys	Arg	Val	Ala	Ala	
	305					310					315					320	
	Ala	Asp	Ile	Glu	Tyr	Glu	Gly	His	Thr	Ile	Lys	Glu	Gly	Asp	Ala	Val	
20					325				330						335		
	Ile	Val	Leu	Leu	Asp	Thr	Ser	Asn	Arg	Asp	Pro	Lys	Val	His	Ala	Glu	
				340					345					350			
25	Pro	Asn	Arg	Leu	Asp	Ile	His	Arg	Ser	Ala	Gly	Asn	His	Leu	Ala	Phe	
			355					360					365				
	Ser	His	Gly	Pro	His	Gln	Cys	Leu	Gly	Lys	His	Leu	Val	Arg	Val	Gln	
		370					375					380					
30	Leu	Glu	Ile	Ala	Leu	Arg	Ala	Val	Ala	Glu	Arg	Leu	Pro	Gly	Leu	Arg	
	385					390					395					400	
	Leu	Asp	Ile	Ala	Lys	Glu	Asp	Ile	Pro	Phe	Arg	Gly	Asp	Ala	Leu	Ser	
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5	Met	Trp	Tyr	Pro	Trp	Thr	Pro	Met	Thr	Glu	Trp	Met	Ala	Arg	Asp	Gln
				20					25					30		
	Leu	Val	Val	Glu	Arg	Ala	Glu	Gly	Cys	Trp	Leu	Ile	Asp	Ala	Asp	Gly
			35					40					45			
10	Lys	Arg	Tyr	Leu	Asp	Gly	Arg	Ser	Ser	Met	Gly	Met	Asn	Leu	His	Gly
		50					55					60				
	His	Gly	Arg	Ser	Glu	Ile	Val	Glu	Ala	Leu	Val	Ala	Gln	Ala	Arg	Lys
15	65					70					75					80
	Ala	Gly	Glu	Thr	Thr	Leu	Tyr	Arg	Val	Ser	His	Pro	Ala	Ala	Val	Glu
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20																
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	Leu	Ala	Ala	Arg	Leu	Ala	Ser	Met	Ala	Pro	Ala	Gly	Leu	Gln	Arg	Val	
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5	Phe	Phe	Ala	Glu	Ser	Gly	Ser	Thr	Ala	Val	Glu	Thr	Ala	Leu	Lys	Ala	
			115					120					125				
	Ala	Tyr	Ala	Tyr	Trp	Val	Ala	Lys	Gly	Glu	Pro	Gln	Arg	Ser	Thr	Phe	
		130					135					140					
10	Val	Ser	Met	Glu	Gly	Gly	Tyr	His	Gly	Glu	Thr	Leu	Gly	Thr	Val	Ser	
	145				150						155				160		
	Leu	Arg	Gly	Thr	Asn	Gly	Glu	Gln	Val	Asp	Met	Ile	Arg	Lys	Thr	Tyr	
				165						170					175		
15	Glu	Pro	Leu	Leu	Phe	Pro	Ser	Leu	Ser	Phe	His	Gln	Pro	His	Cys	Tyr	
			180						185					190			
	Arg	Cys	Pro	Val	Gly	Gln	Ser	Ser	Asp	Ser	Asp	Cys	Gly	Leu	Glu	Cys	
		195						200					205				
20	Thr	Asp	Ser	Leu	Glu	Asn	Leu	Leu	Thr	Arg	Glu	Lys	Gly	Arg	Ile	Ala	
	210					215						220					
	Ala	Val	Ile	Val	Glu	Pro	Arg	Val	Gln	Ala	Leu	Ala	Gly	Val	Ile	Thr	
	225				230						235				240		
25	Ala	Pro	Glu	Gly	His	Leu	Ala	Lys	Val	Ala	Glu	Ile	Thr	Arg	Arg	His	
				245					250					255			
	Gly	Val	Leu	Leu	Ile	Val	Asp	Glu	Val	Leu	Thr	Gly	Trp	Ala	Arg	Thr	
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30	Gly	Pro	Thr	Phe	Ser	Cys	Glu	Ala	Glu	Gly	Val	Thr	Pro	Asp	Leu	Met	
		275					280						285				
	Thr	Val	Gly	Lys	Ala	Leu	Thr	Gly	Gly	Tyr	Leu	Pro	Leu	Ser	Ala	Thr	
	290					295						300					
35	Leu	Ala	Thr	Glu	Glu	Ile	Phe	Gly	Ala	Phe	Arg	Glu	Ser	Val	Phe	Leu	
	305				310						315				320		
	Ser	Gly	Ser	Thr	Tyr	Ser	Gly	Tyr	Ala	Leu	Gly	Ala	Ala	Val	Ala	Leu	
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40	Ala	Ser	Leu	Asp	Leu	Phe	Glu	Lys	Glu	Asp	Val	Pro	Ala	Arg	Ala	Lys	
			340					345						350			
	Ala	Leu	Ala	Asp	Val	Leu	Thr	Thr	Ala	Leu	Glu	Pro	Phe	Arg	Ala	Leu	
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45	Thr	His	Val	Gly	Asp	Val	Arg	Gln	Leu	Gly	Leu	Ile	Ala	Gly	Val	Glu	
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	Leu	Val	Ala	Asp	Arg	Glu	Thr	Arg	Ala	Pro	Tyr	Pro	Pro	Gln	Glu	Arg	
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50	Val	Val	Asp	Arg	Ile	Cys	Thr	Leu	Ala	Arg	Asp	Asn	Gly	Val	Leu	Val	
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	Asn	Ala	Val	Pro	Gly	Asp	Val	Ile	Thr	Met	Leu	Pro	Ser	Pro	Ser	Met	
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Arg Glu Val Thr Glu Glu
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<211> 326

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<213> Streptomyces parvulus Tü4055

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	Arg	Val	Glu	Ala	Cys	Ala	Leu	Asn	His	Leu	Asp	Ile	His	Val	Arg	Asn	
			35					40					45				
10	Gly	Leu	Pro	Gly	Val	Arg	Leu	Glu	Leu	Pro	His	Val	Ser	Gly	Gly	Asp	
	50						55					60					
	Val	Val	Gly	Val	Val	Glu	Gln	Ala	Thr	Asp	Glu	Ala	Gly	Glu	Arg	Leu	
	65					70					75					80	
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	Gly	Glu	His	Tyr	Trp	Gly	Gly	Leu	Ala	Glu	Tyr	Val	Val	Ala	Pro	Ala	
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	Arg	Ala	Arg	Leu	Arg	Pro	Gly	Glu	Ser	Val	Leu	Leu	Phe	Gly	Ala	Thr	
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	Arg	Ile	Ile	Ala	Cys	Ser	Gly	Ser	Pro	Ala	Lys	Leu	Ala	Arg	Leu	Arg	
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35	Arg	Leu	Gly	Val	Ile	Asp	Thr	Ile	Asp	Thr	Gly	Thr	Glu	Asp	Val	Arg	
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	Arg	Arg	Val	Arg	Glu	Leu	Thr	Asp	Gly	Gly	Ala	Asp	Leu	Val	Val	Asp	
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					245					250					255		
	Thr	Asp	Leu	Arg	Tyr	Val	Trp	Ser	Arg	Gln	Leu	Asp	Ile	Leu	Gly	Ser	
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50	Asn	Ala	Trp	His	Arg	Asp	Asp	Leu	His	Thr	Leu	Val	Asp	Leu	Val	Ala	
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	Thr	Asp	Ala	Leu	Glu	Pro	Val	Val	His	Ala	Asp	Phe	Pro	Leu	Ser	Arg
	290						295					300				
5	Ala	Pro	Glu	Ala	Val	Ala	Glu	Leu	Glu	Glu	Arg	Arg	Ala	Phe	Gly	Lys
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5	Ala	Leu	Ala	Met	Gln	Arg	Lys	Ile	Cys	Ala	Gln	Pro	Glu	Glu	Thr	Ala	
				20					25					30			
	Glu	Arg	Val	Phe	Ser	Asp	Ile	Leu	Ser	Val	Ser	Arg	Asp	Thr	Gly	Phe	
			35					40					45				
10	Gly	Arg	Glu	His	Gly	Leu	Ala	Gly	Val	Arg	Thr	Arg	Gln	Glu	Trp	Arg	
	50						55					60					
	Arg	Ala	Val	Pro	Ile	Arg	Thr	Tyr	Asp	Glu	Leu	Ala	Pro	Tyr	Val	Glu	
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	Ala	Phe	Leu	Arg	Thr	Ser	Gly	Ser	Thr	Gly	Arg	Ala	Lys	Leu	Val	Pro	
20				100					105					110			
	Thr	Thr	Asp	His	Trp	Arg	Arg	Val	Tyr	Arg	Gly	Pro	Ala	Leu	Tyr	Ala	
			115					120					125				
25	Gln	Trp	Gly	Leu	Tyr	Phe	Glu	Gln	Ile	Gly	Thr	His	Arg	Leu	Thr	Gly	
	130						135					140					
	Asp	Glu	Val	Leu	Asp	Leu	Ser	Trp	Glu	Pro	Gly	Pro	Ile	Arg	His	Arg	
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	Asp	Pro	Asp	Asp	Trp	Asn	Pro	Pro	Trp	Arg	His	Ala	Arg	Trp	Phe	Thr	
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35	Arg	Asp	Ala	Gly	Ala	Ala	Thr	Met	Ala	Asp	Leu	Leu	Tyr	Gly	Lys	Leu	
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45	Leu	Ile	Gln	Asp	Leu	His	Asp	Gly	His	Gly	Thr	Asp	Arg	Ala	Ala	Arg	
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5	Arg	Pro	Leu	Leu	Thr	Asp	Leu	Trp	Pro	Gly	Leu	Arg	Leu	Leu	Val	Cys	
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	Trp	Asn	Ser	Ala	Ser	Ala	Ala	Leu	Tyr	Gly	Pro	Trp	Leu	Ser	Arg	Leu	
		290					295					300					
10	Ala	Thr	Gly	Val	Ala	Ala	Leu	Pro	Phe	Ser	Thr	Thr	Gly	Thr	Glu	Gly	
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	Ile	Val	Thr	Leu	Pro	Val	Asp	Asp	His	Leu	Ser	Ala	Gly	Pro	Leu	Ala	
					325					330					335		
15	Val	Asp	Gln	Gly	His	Phe	Glu	Phe	Val	Pro	Trp	Gln	Asp	Leu	Asp	Asp	
				340					345					350			
	Gly	Ser	Pro	Leu	Pro	Glu	Asp	Thr	Pro	Thr	Leu	Gly	Tyr	Asp	Glu	Leu	
20			355					360					365				
	Glu	Leu	Gly	Ala	Asp	Tyr	Arg	Leu	Val	Met	Ser	Gln	Ala	Asn	Gly	Leu	
		370					375					380					
25	Tyr	Arg	Tyr	Asp	Val	Gly	Asp	Val	Tyr	Arg	Val	Val	Gly	Ala	Val	Gly	
	385					390					395					400	
	Ala	Thr	Pro	Arg	Leu	Glu	Phe	Leu	Gly	Arg	Ala	Gly	Phe	Gln	Ser	Ser	
					405					410					415		
30	Phe	Thr	Gly	Glu	Lys	Leu	Thr	Glu	Ser	Asp	Val	His	Thr	Ala	Val	Met	
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	Arg	Val	Leu	Gly	Ser	Glu	Arg	Thr	Asp	His	Pro	His	Phe	Ser	Gly	Ile	
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	Asp	Ala	His	Gly	Thr	Leu	Asn	Val	Gln	Asp	Thr	Ala	Arg	Arg	Ile	Asp	
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	Ala	Thr	Leu	Gln	Glu	Val	Asn	Val	Glu	Tyr	Ala	Asp	Lys	Arg	Arg	Ser	
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45	Gly	Arg	Leu	Arg	Pro	Leu	Gln	Ile	Leu	Pro	Leu	Val	Pro	Gly	Ala	Phe	
				500					505					510			
	Gly	Gln	Ile	Ala	Glu	Arg	Arg	Phe	Arg	Gln	Gly	Thr	Ala	Gly	Ala	Gln	
			515					520					525				
50	Ile	Lys	His	His	Trp	Leu	Gln	Lys	Asp	Ser	Ala	Phe	Leu	Asp	Thr	Leu	
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<212> PRT

<213> Streptomyces parvulus Tü4055

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	Ser	Leu	Trp	Thr	Tyr	Gln	Arg	Val	Leu	Gly	Ala	Pro	Asp	Asp	Ser	Trp
			35					40					45			
10	Gly	Glu	Ala	Asn	Arg	Ser	Val	His	Asp	Pro	Leu	Thr	Thr	Leu	Ala	Phe
		50					55					60				
	Leu	Ala	Ala	His	Thr	Thr	Gly	Ile	Arg	Leu	Gly	Val	Ala	Val	Leu	Ile
	65					70					75				80	
15	Met	Pro	Leu	His	Thr	Pro	Ala	Val	Leu	Ala	Lys	Gln	Leu	Thr	Thr	Leu
				85						90				95		
	Asp	Leu	Leu	Ser	Gly	Gly	Arg	Leu	Asp	Val	Gly	Leu	Gly	Asn	Gly	Trp
			100					105						110		
20	Ala	Ala	Glu	Glu	Tyr	Ala	Ala	Ala	Gly	Val	Thr	Pro	Thr	Gly	Leu	Ser
			115					120					125			
	Arg	Arg	Ala	Glu	Asp	Phe	Leu	Ala	Cys	Leu	Arg	Ala	Leu	Trp	Gly	Glu
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30	Arg	Phe	Asp	Pro	Lys	Pro	Ala	Gln	Ser	Pro	His	Pro	Pro	Leu	Leu	Leu
					165				170						175	
	Gly	Gly	Ala	Ala	Pro	Gly	Ala	Leu	Arg	Arg	Ala	Gly	Arg	Leu	Cys	Asp
			180					185						190		
35	Gly	Trp	Ile	Ala	Ser	Ser	Lys	Ala	Gly	Pro	Ala	Ala	Ile	Arg	Asp	Ala
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	Ile	Thr	Val	Val	Arg	Asp	Ser	Ala	Glu	Arg	Thr	Gly	Arg	Asp	Pro	Ala
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40	Thr	Leu	Arg	Phe	Val	Cys	Arg	Ala	Pro	Val	Arg	Leu	Arg	Thr	Arg	Ser
	225					230					235					240
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	Asp	Leu	Ala	Ala	Leu	Ala	Asp	Thr	Gly	Leu	Thr	Glu	Ile	Phe	Leu	Asp
			260						265					270		
50	Pro	Asn	Phe	Asp	Pro	Glu	Ile	Gly	Ser	Pro	Asp	Ala	Pro	Thr	Gly	Asp
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Leu Leu Ala Pro Val Glu Pro Ser Lys Val Leu Val Ala Gly Arg Asn
 50 55 60

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Tyr Gly Asp Val Val Thr Pro Asp Leu Val Val Phe Met Lys Pro Ser
 65 70 75 80

Thr Ser Val Val Gly Pro Arg Ser Thr Val Leu Leu Pro Ala Glu Ala
 85 90 95

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Lys Gln Val Arg Tyr Glu Gly Glu Leu Ala Val Val Ile Gly Arg Arg
 100 105 110

Cys Lys Asp Val Pro Glu Asp Thr Ala Asp Gln Ala Val Phe Gly Tyr
 115 120 125

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Thr Cys Ala Asn Asp Val Thr Ala Trp Asp Val Gly Glu Pro Lys Gly
 130 135 140

His Trp Thr Lys Ala Lys Ser Phe Asp Thr Phe Cys Pro Leu Gly Pro
 145 150 155 160

Trp Ile Arg Thr Asp Leu Asp Pro Ala Asp Leu Val Leu Arg Thr Thr
 165 170 175

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Val Asn Gly Thr Leu Arg Gln Asp Gly Ser Thr Lys Glu Met Asn Arg
 180 185 190

Asn Val Arg Ala Leu Val Ser Arg Cys Ser Ser Leu Met Thr Leu Leu
 195 200 205

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Pro Gly Asp Val Ile Leu Thr Gly Thr Pro Ala Gly Ala Gly Val Leu
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Arg Pro Gly Asp Glu Val Val Val Glu Ile Asp Gly Ile Gly Ser Leu
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10	Pro	Arg	Asn	Gly	Pro	Asn	Ala	Ile	Val	Val	Val	Arg	Asp	Pro	Ser	Gly
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15																
20																
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5	Ala	Val	Ala	Leu	Ser	Ser	Glu	Asp	Gly	Leu	Thr	Val	Leu	Arg	His	Ser	
	65					70					75					80	
	Thr	Ala	His	Val	Leu	Ala	Gln	Ala	Val	Gln	Gln	Leu	Trp	Pro	Glu	Ala	
					85					90					95		
10	Arg	Leu	Gly	Ile	Gly	Pro	Pro	Ile	Glu	Asn	Gly	Phe	Tyr	Tyr	Asp	Phe	
				100					105					110			
	Asp	Val	Glu	Arg	Pro	Phe	Gln	Pro	Glu	Asp	Leu	Glu	Arg	Val	Glu	Gln	
			115					120					125				
15	Arg	Met	Lys	Glu	Ile	Ile	Lys	Ser	Gly	Gln	Arg	Phe	Cys	Arg	Arg	Glu	
		130					135					140					
	Phe	Pro	Asp	Arg	Glu	Ala	Ala	Arg	Ala	Glu	Leu	Ala	Lys	Glu	Pro	Tyr	
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25	Arg	Thr	Gly	Asp	Val	Cys	Trp	Ser	Asp	Leu	Cys	Arg	Gly	Pro	His	Leu	
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30	Ala	Tyr	Trp	Arg	Gly	Ser	Glu	Lys	Asn	Pro	Gln	Leu	Gln	Arg	Ile	Tyr	
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					245					250					255		
35	Leu	Glu	Glu	Ala	Ala	Lys	Arg	Asp	His	Arg	Arg	Ile	Gly	Glu	Glu	Leu	
				260					265					270			
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			275					280					285				
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45	Thr	Gln	Glu	Asp	Leu	Tyr	Tyr	Leu	Ser	Gly	His	Leu	Pro	Tyr	Tyr	Ala	
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				340				345					350				
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	Lys Asp Gln Phe Leu Glu Val Met Arg Met His Ala Asp Tyr Tyr Arg	420		425		430	
10	Thr Leu Gly Ile Ser Asp Phe Tyr Met Val Leu Ala Leu Arg Asp Ser	435		440		445	
	Ala Asn Lys Asp Lys Tyr His Asp Asp Glu Gln Met Trp Glu Asp Ala	450		455		460	
15	Glu Arg Ile Thr Arg Glu Ala Met Glu Glu Ser Asp Ile Pro Phe Gln	465		470		475	480
	Ile Asp Leu Gly Gly Ala Ala His Tyr Gly Pro Lys Val Asp Phe Met	485		490		495	
20	Ile Arg Ala Val Thr Gly Lys Glu Phe Ala Ala Ser Thr Asn Gln Val	500		505		510	
	Asp Leu Tyr Thr Pro Gln Arg Phe Gly Leu Thr Tyr His Asp Ser Asp	515		520		525	
25	Gly Thr Glu Lys Pro Val Val Val Ile His Arg Ala Pro Leu Gly Ser	530		535		540	
	His Glu Arg Phe Thr Ala Tyr Leu Thr Glu His Phe Ala Gly Ala Phe	545		550		555	560
	Pro Val Trp Leu Ala Pro Glu Gln Val Arg Ile Ile Pro Ile Val Glu	565		570		575	
35	Glu Leu Thr Asp Tyr Ala Glu Glu Val Arg Asp Met Leu Leu Asp Ala	580		585		590	
	Asp Val Arg Ala Asp Val Asp Ala Gly Asp Gly Arg Leu Asn Ala Lys	595		600		605	
40	Val Arg Ala Ala Val Thr Arg Lys Ile Pro Leu Val Val Val Val Gly	610		615		620	
	Arg Arg Glu Ala Glu Gln Arg Thr Val Thr Val Arg Asp Arg Ser Gly	625		630		635	640
45	Glu Glu Thr Pro Met Ser Leu Glu Lys Phe Val Ala His Val Thr Gly	645		650		655	
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<213> Streptomyces parvulus Tü4055

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15     Gly Ala Trp Val Trp Ala Thr Val Phe Asp Arg Tyr Ala Ser Arg Thr
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      Tyr Asp Asp Ala Gln Asp Val Asp Ala Ile His Asp Ala Ala Gly Leu
      50          55          60

20     Val Met Ala Gly Ala Gly Phe Asp Ile Leu Ala Ala Val Leu Ala Ile
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				20					25					30			
	Arg	Ala	Ala	Leu	Arg	Arg	Leu	Glu	Ala	Glu	Leu	Leu	Gly	Arg	Gly	His	
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10	Ala	Val	Ser	Ala	Pro	Leu	His	Ala	Val	Leu	Ala	Ser	Leu	Asp	Ser	Glu	
		50					55					60					
	Glu	Leu	Ala	Ala	Ala	His	Val	Arg	Leu	Val	Gly	Leu	Val	Asp	Asp	Leu	
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					85					90					95		
	Thr	Val	Pro	Arg	Asp	Thr	Glu	Ala	Leu	Tyr	Val	Asp	Arg	Val	Phe	Ala	
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	Phe	Leu	Leu	Gln	Gln	Pro	Glu	Gln	Pro	Cys	Val	Leu	Cys	Gly	Glu	Ala	
			115					120					125				
25	Arg	Thr	Val	Leu	Pro	Val	Ser	Pro	Cys	Ala	His	Leu	Val	Cys	Arg	Leu	
		130					135					140					
	Cys	Trp	Asp	Gly	Ser	Asp	Tyr	Ala	Gly	Cys	Pro	Leu	Cys	His	Arg	Arg	
	145					150					155					160	
30	Ile	Asp	Gly	Asp	Asp	Pro	Phe	Leu	Arg	Pro	Val	Arg	Ala	Val	Gly	Ala	
					165					170					175		
	Ala	Arg	Ala	Thr	Val	Pro	Gly	Pro	Leu	Arg	Leu	Leu	Arg	Leu	Gly	Thr	
				180					185					190			
35	Asp	Met	Thr	Ala	Asp	Ala	Thr	Thr	Ala	Val	Asp	Ala	Leu	Leu	Ala	Arg	
			195					200					205				

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	Arg	Thr	Pro	Leu	Ser	Pro	Gln	Asp	Arg	Asp	Asp	Leu	Leu	Thr	Leu	Leu	
	210						215					220					
5	Pro	Leu	Thr	Pro	Ala	Gly	Arg	Gly	Asp	Leu	Pro	Gln	Asp	Ile	Pro	Val	
	225					230					235					240	
	Arg	Glu	Thr	Lys	Ala	Leu	Val	Leu	Gly	Ala	Leu	Val	Arg	Arg	Ala	Pro	
					245					250					255		
10	Ser	Arg	Pro	Ala	Leu	Arg	Arg	Leu	Leu	Ala	Glu	Arg	Leu	Thr	Thr	Ala	
				260					265					270			
	Thr	Asp	Val	Leu	Arg	Leu	Leu	Ala	Val	Leu	Ser	Gly	Gly	Asp	Ala	Gly	
			275					280					285				
15	Leu	Val	Thr	Pro	Ala	Arg	Phe	Thr	Asn	Val	Pro	Arg	Ser	Leu	Arg	Arg	
	290						295					300					
	Asp	Leu	Leu	Ala	Val	Leu	Asp	Gly	Leu	Pro	Ala	Pro	Tyr	Leu	Val	Glu	
	305					310					315					320	
20	Asp	Met	Leu	Arg	His	Pro	Thr	Ala	Trp	Lys	Arg	Ala	Ala	Glu	Val	Leu	
					325					330					335		
	His	Pro	Phe	Glu	Gly	His	Thr	Arg	His	Pro	Arg	Ala	Ala	Leu	Ala	Thr	
				340					345					350			
25	Ala	Val	Leu	Arg	Ala	Thr	Pro	Leu	Asp	Pro	Asp	Thr	Ala	Phe	Gly	Ala	
			355					360					365				
	Ala	Leu	Leu	Thr	Thr	Ala	Ala	Ala	His	Pro	Asp	Ala	Val	Arg	Pro	Asp	
	370					375						380					
30	Gly	Thr	Arg	Val	Arg	Pro	Ala	Thr	Trp	Ala	Gly	Arg	Leu	Glu	Gln	Ala	
	385					390					395					400	
	Met	Ala	Glu	Gly	Asp	Ala	Ala	Arg	Ala	Ala	Ala	Leu	Ala	Gly	Glu	Arg	
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35	Pro	Gly	Glu	Leu	Val	Arg	Arg	Leu	Asp	Val	Leu	Leu	Arg	Leu	His	Thr	
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	Asp	Glu	Ala	Leu	Val	Pro	Glu	Leu	Glu	Lys	Ala	Leu	Arg	His	Gly	Leu	
			435					440					445				
40	Pro	Lys	Val	Gly	Pro	Gly	Pro	Leu	Leu	Ser	Ala	Leu	Gly	Ala	Leu	Arg	
		450					455					460					
	Thr	Arg	Thr	Glu	Asp	Arg	Thr	Gly	Thr	Arg	Arg	Val	Phe	Phe	Pro	Arg	
	465					470					475					480	
45	Gly	Asp	Val	Thr	Arg	Ala	Leu	Ser	Val	Pro	Glu	Arg	Arg	Pro	Ala	Leu	
					485					490					495		
	Pro	Ala	Gly	Pro	Val	Ser	Glu	Val	Val	Ala	Leu	Leu	Glu	Gly	Glu	Leu	
				500					505					510			
50	Leu	Arg	Arg	Phe	Ala	Ala	Gly	Arg	Pro	Tyr	Glu	Leu	Ser	Val	Leu	Asp	
			515					520					525				
	Ala	Gly	Leu	Thr	Asp	Leu	Thr	Val	Pro	Phe	Thr	Glu	Arg	Thr	Ala	Ala	
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	545		550		555		560
	Gly Ser Val Leu Arg Leu Phe Leu His Trp Thr Glu Pro Arg Gly Asn						
		565			570		575
5	Arg Thr Asp Leu Asp Leu Ser Val Ala Phe Phe Asp Ala Glu Trp Thr						
		580		585			590
	Phe Thr Gly Leu Cys Asp Tyr Thr Asn Leu Val His Gly Pro Asp Ala						
10		595		600			605
	Ala Ile His Ser Gly Asp Leu Thr Ser Ala Pro Ala Pro Arg Gly Ala						
		610		615			620
	Thr Glu Tyr Val Asp Leu Asp Leu Glu Arg Leu Ala Arg Arg Gly Asp						
15		625		630		635	640
	Thr Tyr Ala Val Pro Leu Val Phe Ser Tyr Asn Asn Val Pro Phe Glu						
		645		650			655
	Glu Leu Pro Asp Ala Phe Ala Gly Phe Met Ala Leu Pro Ala Glu Gly						
20		660		665			670
	Pro Arg Asp Ala Thr Tyr Asp Pro Arg Thr Val Arg Gln Arg Phe Asp						
		675		680			685
	Leu Ala Gly Asp Ser Lys Val Cys Leu Pro Met Ile Val Asp Leu Ala						
25		690		695			700
	Arg Arg Arg Ala Leu Trp Thr Asp Thr His Leu Pro Ser Ala Gly Gly						
		705		710		715	720
30	Phe Gln Ser Ile Gly Ser His Gly Gly Gly Glu Leu Ala Ala Val Ala						
		725		730			735
	Gly Asp Leu Trp Gln Gln Phe Thr Ser Gly Gly Arg Ala Thr Leu Trp						
35		740		745			750
	Asp Leu Ala Val Leu Arg Ala Ala Ala Leu Ser Pro Glu Val Ala Val						
		755		760			765
	Val Ser Arg Glu Pro Glu Pro Ala Val Leu Arg Tyr Arg Arg Arg Ala						
40		770		775			780
	Ala Glu Ser Glu Ala Ala Phe Ala Val Arg Val Ala Ser His Lys Asp						
		785		790		795	800
	Ala Glu Glu Arg Leu Ala His Thr Asp Pro Asp Ser Ala Ala Ala Gly						
45		805		810			815
	Leu Ala Ala Gly Arg Arg Val Phe Leu Ala Thr Val His Gly Asp Val						
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	Arg Pro Pro Gly Ala Ser Gly Thr Ser Tyr Arg Leu Phe Pro Gly Ala						
50		835		840			845
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His His Ser Arg Arg Val Tyr Leu Phe Gly Ser Leu Gln Ala Ala Ala
 35 40 45

Leu Gly Ile Arg Pro Asp Pro Glu Leu Leu Tyr Ile Ala Ala Leu Phe
 50 55 60

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His Asp Thr Gly Leu Val Pro Pro Tyr Arg Gly Asp Asp Gln Arg Phe
 65 70 75 80

Glu Met Asp Gly Ala Asp Gln Ala His Ala Phe Leu Leu Ala His Gly
 85 90 95

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Ile Pro Glu Ala Asp Ala Asp Thr Val Trp Thr Ala Val Ala Leu His
 100 105 110

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Thr Thr Pro Glu Val Pro Tyr Arg Met Ala Pro Glu Ile Ala Ala Thr
 115 120 125

Thr Ala Gly Val Glu Thr Asp Val Leu Gly Leu Arg Leu Gly Asn Leu
 130 135 140

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Thr Arg Ala Gln Ile Asp Ala Val Thr Ala Ala His Pro Arg Pro Asp
 145 150 155 160

Phe Lys Lys Gln Ile Leu Arg Ala Phe Thr Glu Gly Phe Glu His Arg
 165 170 175

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Pro Ala Thr Thr Phe Gly Thr Val Asn Ala Asp Val Leu Glu His Phe
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Ala Pro Gly Phe Arg Arg Thr Asp Phe Val Glu Val Ile Glu Asn Ser
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Ala Trp Pro Glu
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5	Lys	Met	Leu	Asp	Leu	Ser	Gly	Pro	Ala	Glu	Val	Phe	Ala	Glu	Ala	Asn
				20					25					30		
	Arg	Phe	Gly	Ala	Arg	Tyr	Arg	Leu	Gly	Val	Val	Ser	Pro	Asp	Gly	Ala
			35					40					45			
10	Pro	Val	Arg	Ser	Ser	Ile	Gly	Leu	Leu	Val	Pro	Ala	Glu	Ala	Asp	Ala
		50					55					60				
15																
20																
25																
30																
35																
40																
45																
50																
55																

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	Arg	Ser	Ala	Gly	Pro	Pro	Asp	Thr	Leu	Val	Val	Val	Gly	Gly	Asp	Ala	
	65					70					75					80	
5	Leu	Pro	Gly	Ser	Pro	Val	Asp	Pro	Arg	Leu	Ile	Asp	Ala	Ala	Lys	Ala	
					85					90					95		
	Leu	Ala	Ala	Arg	Ala	Gly	Arg	Val	Ala	Ser	Val	Cys	Thr	Gly	Ala	Phe	
				100					105					110			
10	Val	Leu	Gly	Ala	Ala	Gly	Leu	Leu	Glu	Gly	Arg	Arg	Ala	Thr	Thr	His	
			115					120					125				
	Trp	Gln	His	Thr	Thr	Ala	Leu	Ala	Arg	Arg	Cys	Pro	Ser	Thr	Arg	Val	
15		130					135					140					
	Glu	Pro	Asp	Ala	Ile	Phe	Val	Lys	Asp	Gly	Ala	Thr	Tyr	Thr	Ser	Ala	
	145					150					155					160	
20	Gly	Val	Thr	Ala	Gly	Ile	Asp	Leu	Ala	Leu	Ala	Leu	Leu	Glu	Glu	Asp	
					165					170					175		
	His	Gly	Pro	Asp	Leu	Ala	Arg	Arg	Val	Ala	Arg	Ser	Leu	Val	Val	Tyr	
				180					185					190			
25	Leu	Gln	Arg	Ala	Gly	Gly	Gln	Ser	Gln	Phe	Ser	Ala	Ser	Leu	Arg	Gly	
			195					200					205				
	Pro	Ala	Pro	Arg	Thr	Pro	Val	Leu	Arg	Gln	Val	Gln	Asp	Ala	Val	Gln	
		210					215					220					
30	Ala	Asp	Pro	Ala	Ala	Asp	His	Ser	Leu	Ala	Ala	Leu	Ala	Ala	Arg	Val	
	225					230					235					240	
	Arg	Val	Ser	Pro	Arg	His	Leu	Thr	Arg	Met	Phe	Arg	Ala	Glu	Leu	Asp	
35					245					250					255		
	Val	Thr	Pro	Val	Lys	Tyr	Val	Glu	Leu	Ile	Arg	Phe	Asp	Ile	Ala	Lys	
				260					265					270			
40	Ala	Leu	Leu	Asp	Ser	Gly	His	Asn	Ala	Thr	Glu	Ala	Ala	Ala	Leu	Ser	
			275					280					285				
	Gly	Phe	Pro	Ser	Tyr	Glu	Ser	Leu	Arg	Arg	Ala	Phe	Ala	Arg	His	Leu	
		290					295					300					
45	Gly	Leu	Ser	Pro	Thr	Arg	Tyr	Arg	Gln	Arg	Phe	Ala	Thr	Thr	Val	Pro	
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5	Trp	Gly	Pro	Arg	Asp	Ala	Pro	Pro	Ile	Val	Phe	His	His	Gly	Trp	Pro	
				20					25					30			
	Leu	Thr	Ala	Asp	Asp	Trp	Asp	Asn	Gln	Met	Leu	Phe	Phe	Leu	Ser	His	
10																	
				35					40					45			
	Gly	Tyr	Arg	Val	Ile	Ala	His	Asp	Arg	Arg	Gly	His	Gly	Arg	Ser	Gly	
		50					55					60					
15	Gln	Pro	Ser	Thr	Gly	His	Glu	Met	Asp	Thr	Tyr	Ala	Ala	Asp	Val	Ala	
	65					70					75					80	
	Ala	Leu	Thr	Glu	Ala	Leu	Asp	Leu	Arg	Asp	Ala	Val	His	Ile	Gly	His	
20					85					90					95		
	Ser	Thr	Gly	Gly	Gly	Glu	Val	Ala	Arg	Tyr	Val	Ala	Arg	Ala	Glu	Pro	
				100					105					110			
	Gly	Arg	Val	Ala	Lys	Ala	Val	Leu	Val	Gly	Ala	Val	Pro	Pro	Val	Met	
25				115				120					125				
	Val	Lys	Ser	Asp	Ala	Asn	Pro	Gly	Gly	Thr	Pro	Ile	Glu	Val	Phe	Asp	
		130					135					140					
30	Gly	Phe	Arg	Thr	Ala	Leu	Ala	Ala	Asn	Arg	Ala	Gln	Phe	Tyr	Ile	Asp	
	145					150				155						160	
	Val	Pro	Ser	Gly	Pro	Phe	Tyr	Gly	Phe	Asn	Arg	Glu	Gly	Ala	Lys	Val	
				165						170					175		
35	Ser	Gln	Gly	Leu	Ile	Asp	Asn	Trp	Trp	Arg	Gln	Gly	Met	Ser	Gly	Ala	
				180					185					190			
	Ala	Asn	Ala	His	Tyr	Glu	Cys	Ile	Lys	Ala	Phe	Ser	Glu	Thr	Asp	Phe	
40				195				200					205				
	Thr	Glu	Asp	Leu	Lys	Ala	Ile	Asp	Val	Pro	Val	Leu	Val	Ala	His	Gly	
		210					215					220					
	Thr	Asp	Asp	Gln	Val	Val	Pro	Tyr	Ala	Asp	Ser	Ala	Pro	Leu	Ser	Val	
45		225				230					235					240	
	Lys	Leu	Leu	Lys	Asn	Gly	Thr	Leu	Lys	Ser	Tyr	Glu	Gly	Leu	Pro	His	
				245						250					255		
50	Gly	Met	Leu	Ser	Thr	His	Pro	Glu	Val	Val	Asn	Pro	Asp	Leu	Leu	Asp	
				260					265					270			
	Phe	Val	Arg	Ser													
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<213> Streptomyces parvulus Tü4055

<400> 27

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10     His Val Thr Gln Gln Ala Pro Val Ala Thr Asp Glu Arg Arg Val Phe
      20          25          30

15     Val Asp Lys Gln Thr Pro Gly Ala Tyr Lys Ala Phe Val Ala Ala Ala
      35          40          45

15     Glu Ser Val Arg Glu Ala Ala Ala Ala Gly Leu Asp Arg Leu Leu
      50          55          60

20     Val Glu Leu Val Asn Ile Arg Val Ser Gln Leu Asn Ala Cys Ala Tyr
      65          70          75          80

25     Cys Leu Ser Leu His Thr Arg Ala Ala Leu Arg Ala Gly Glu Thr Thr
      85          90          95

25     Gln Arg Leu Ala Val Leu Pro Ala Trp Arg Asp Thr Glu Leu Phe Thr
      100         105         110

30     Ala Arg Glu Arg Ala Ala Leu Ala Leu Ala Glu Ala Thr Thr Arg Pro
      115         120         125

30     Ala Asp Ala Ala Ala Gln Ser Ala Ala Tyr Ala Gln Ala Arg Gly Val
      130         135         140

35     Leu Ser Asp Asp Glu Val Ser Ala Val Ile Trp Val Ala Ile Ser Ile
      145         150         155         160

35     Asn Ala Phe Asn Arg Val Ser Val Leu Ser Lys His Pro Val Arg Gly
      165         170         175

40     Ala Ala Pro Ala Pro Val Ser Pro Ala
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5	Leu	Glu	Asp	Glu	Val	Pro	Ala	Ala	Gly	Val	Glu	Val	Leu	Thr	Ala	Arg	
				20					25					30			
	Asp	Val	Pro	Leu	Gly	Gly	Pro	Arg	Ala	Met	Thr	Val	Arg	Arg	Thr	Leu	
			35					40					45				
10	Pro	Gln	Arg	Ala	Arg	Thr	Leu	Ile	Gly	Ala	Trp	Cys	Phe	Ala	Asp	His	
		50					55					60					
	Tyr	Gly	Pro	Asp	Asp	Val	Ala	Ala	Ser	Gly	Gly	Met	Asp	Val	Ala	Pro	
	65					70					75					80	
15	His	Pro	His	Ile	Gly	Leu	Gln	Thr	Val	Ser	Trp	Leu	Phe	Ser	Gly	Glu	
					85					90					95		
	Ile	Glu	His	Arg	Asp	Ser	Leu	Gly	Thr	His	Ala	Phe	Val	Arg	Pro	Gly	
20				100					105					110			
	Glu	Leu	Asn	Leu	Met	Thr	Gly	Gly	Phe	Gly	Ile	Ala	His	Ser	Glu	Val	
			115					120					125				
25	Ser	Thr	Pro	Asp	Thr	Thr	Val	Leu	His	Gly	Val	Gln	Leu	Trp	Val	Ala	
		130					135					140					
	Leu	Pro	Glu	Glu	His	Arg	Asp	Thr	Gly	Arg	Asp	Phe	Gln	His	His	Ala	
	145					150					155					160	
30	Pro	Ala	Pro	Val	Ala	Phe	Asp	Gly	Gly	Thr	Ala	Arg	Val	Phe	Leu	Gly	
					165					170					175		

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	Ser	Leu	Ala	Gly	Asp	Thr	Ser	Pro	Val	Ser	Thr	Phe	Thr	Pro	Leu	Leu
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5	Gly	Ala	Glu	Leu	Thr	Leu	Val	Pro	Gly	Gly	Thr	Ala	Thr	Leu	Asp	Val
			195					200					205			
	Asp	Pro	Gly	Phe	Glu	His	Gly	Val	Leu	Val	Asp	Ser	Gly	Asp	Val	Arg
		210					215					220				
10	Val	Glu	Gly	Ala	Val	Val	Arg	Pro	Ala	Glu	Leu	Gly	Tyr	Val	Ala	Pro
	225					230					235					240
	Gly	Arg	Ala	Thr	Leu	Thr	Leu	Thr	Asn	Glu	Ser	Ala	Ala	Pro	Ala	Arg
				245						250					255	
15	Leu	Ile	Leu	Leu	Gly	Gly	Pro	Pro	Phe	Pro	Glu	Glu	Ile	Ile	Met	Trp
			260						265					270		
	Trp	Asn	Phe	Ile	Gly	Arg	Ser	His	Asp	Glu	Ile	Val	Arg	Ala	Arg	Glu
20			275					280					285			
	Asp	Trp	Met	Lys	Gly	Asp	Arg	Phe	Gly	Glu	Val	His	Gly	Tyr	Asp	Gly
		290					295					300				
25	Ala	Pro	Leu	Pro	Ala	Pro	Glu	Leu	Pro	Asn	Ala	Pro	Leu	Lys	Pro	Arg
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				20					25					30			
	Asp	Asn	Pro	Asn	Val	Arg	Ile	Arg	Asp	Ile	Ala	Ala	His	Cys	Arg	Leu	
			35					40					45				
10	Thr	Glu	Arg	Ala	Val	Gln	Arg	Ile	Ile	Ser	Asp	Leu	Glu	Gln	Asp	Gly	
		50					55					60					
	Tyr	Leu	Ser	His	Thr	Arg	Asp	Gly	Arg	Ser	Asn	Ile	Tyr	Arg	Val	Glu	
	65					70					75					80	
15	Pro	Asp	Lys	Val	Leu	Arg	His	Pro	Ala	Glu	Ala	Gly	Leu	Thr	Val	Ala	
					85					90					95		
	Ala	Leu	Leu	Ser	Leu	Leu	Val	Arg	Asp	Glu	Thr	Asp	His	Gly	Arg	Ser	
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	Ala	Gly	Pro	Gly	Ser	Arg	Pro	Ala	Arg	Ser	Ser	Ala	Ala	Arg			
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				20					25					30			
	His	Gly	Ala	Trp	Val	Val	Ser	Ala	Arg	Gly	Ala	Tyr	Asp	Met	Asn	Ser	
40			35					40					45				
	Val	Glu	Pro	Leu	Ala	Asp	Ala	Leu	Lys	Asp	Ala	Ala	Glu	Lys	Ser	Pro	
		50					55					60					
45	Lys	Val	Val	Leu	Asp	Ala	Ser	Gly	Ile	Thr	Phe	Ala	Asp	Ser	Thr	Leu	
	65				70						75					80	
	Leu	Ser	Leu	Leu	Ile	Leu	Thr	His	Gln	Ala	Thr	Asp	Phe	Arg	Val	Ala	
					85					90					95		
50	Ala	Pro	Thr	Trp	Gln	Val	Met	Arg	Leu	Met	Gln	Leu	Thr	Gly	Val	Asp	
				100					105					110			
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10     Thr Ser Arg Leu Leu Glu Asp Ala Thr Tyr Thr Leu Cys Val Met Thr
      35          40          45

      Gly Ala Arg Thr Ala Glu Gln Ala Leu Arg Thr Ala Asp Glu Leu Leu
15     50          55          60

      Ala Gln Phe Ala Glu Arg Thr Ala Ala Pro Val Glu Asp Glu Ala Leu
      65          70          75          80

20     Ala Ala

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      Ala Thr Glu Ile Pro Ala Pro Gln Trp Arg Asp Leu Phe Leu Ala Pro
      20          25          30

35     Asp Trp Gly Gly Thr Asp Glu Gln Val Ile Val Ala Glu Glu Ala Arg
      35          40          45

40     Gly Pro Glu His Phe Thr Gly Ala Arg Arg Pro Arg Gly Gly Arg Arg
      50          55          60

      Ser Ser Arg Arg Ala Ala
      65          70

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5	Gly	Gly	Asn	Val	Ala	Val	Asp	Met	Gly	Glu	Thr	Gly	Leu	Asp	Gly	Ser	
				20					25					30			
	Ser	Thr	Gln	Arg	Ala	Pro	Glu	Gly	Ala	Asp	Pro	Arg	Ala	Ala	Ser	Val	
			35					40					45				
10	Thr	Tyr	Arg	Arg	Glu	Ala	Leu	Arg	Ile	Ala	Asp	Ala	Arg	His	Phe	Ala	
	50						55					60					
	Thr	Asp	Tyr	Leu	Thr	Arg	Ser	Gln	Arg	Asp	Leu	Arg	Ser	Pro	Val	Pro	
15	65					70					75					80	
	Glu	Arg	Ala	Ser	Glu	Ala	Val	His	Leu	Val	Val	Ser	Glu	Leu	Ile	Thr	
					85					90					95		
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 50 55 60
 Gly Glu Asp Val Asp His Ser Val His Glu His His Glu Ala Asn Glu
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 Asp Asp Lys Leu Glu Glu Leu Val Thr Ala Val Asn His His Ala Asp
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	Thr	Pro	Pro	Lys	Lys	Trp	Ala	Ala	Gly	Val	Pro	Ala	Val	Val	His	Ala
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	Leu	Leu	Thr	Met	Asn	Gln	Val	Gly	Gly	Ile	Asp	Cys	Pro	Gly	Cys	Ala	65	70	75	80
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	Gly	Val	Leu	Ala	Glu	Glu	Leu	Lys	Ser	Leu	Ala	Ser	Pro	Asp	Glu	Ala	165	170	175	
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50	Arg	Glu	Glu	Ile	Glu	Lys	Val	Arg	Asp	Glu	Val	Leu	Asp	Ser	Glu	Arg	370	375	380	
	Val	Val	Val	Cys	Trp	Ala	Met	Gly	Ile	Thr	Gln	His	Lys	His	Gly	Val	385	390	395	400
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 40 His Arg Gly Glu Val Glu Phe Arg Pro Thr Glu Ser Gly Gly Thr Ala
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      145             150             155             160

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	Glu	Ala	Val	Asp	Ala												

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5	Gln	Pro	Phe	Ala	Gly	Asp	Lys	Asp	Ser	Gly	Val	Gly	Val	Ala	Gly	Gly
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	Arg	His	Val	Cys	Glu	Gly	Asp	Ala	Val	Pro	Gly	Leu	Leu	Ile	Pro	Gln	
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	Ser	Ala	Leu	Ala	Phe	Glu	Ala	Lys	Leu	Glu	Lys	Asp	Leu	Leu	Ala	Tyr	
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	225					230					235					240	
	Leu	Leu	Arg	Leu	Phe	Asp	Lys	Gly	Pro	Arg	Pro	Asp	Ser	Ala	Gly	Glu	
					245					250					255		
50	Leu	Ala	Ala	Arg	Gly	Trp	Ser	Thr	Ser	Met	His	Thr	Pro	Phe	Val	Phe	
				260					265					270			
	Thr	His	Arg	Tyr	Gly	Arg	Gly	Pro	Leu	Pro	Glu	Pro	Asn	Asp	Ala	Leu	
			275					280					285				
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Ala Lys Ala Ala Val Arg Ala Ala Phe Arg Arg Tyr Trp Thr Thr Leu
 50 55 60

20

Ala Thr Glu Leu Asp Ala Asp Gly Asp Gly Val Ile Thr Val Glu Glu
 65 70 75 80

Phe Arg Pro Phe Val Leu Asp Pro Glu Arg Phe Gly Pro Thr Ile Ala
 85 90 95

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Glu Phe Ala Arg Ala Leu Ser Ala Leu Gly Asp Pro Asp Gly Asp Gly
 100 105 110

Leu Ile Glu Arg Pro Leu Phe Val Ala Leu Met Lys Ala Ile Gly Phe
 115 120 125

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Glu Glu Ala Asn Ile His Ala Leu Phe Asp Ala Phe Ala Pro Asp Ala
 130 135 140

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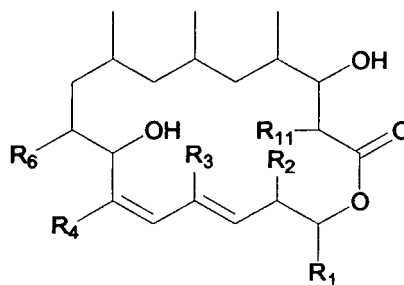
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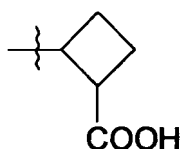
Claims

1. A compound of formula 1 or a pharmaceutically acceptable salt thereof, wherein:

Formula 1

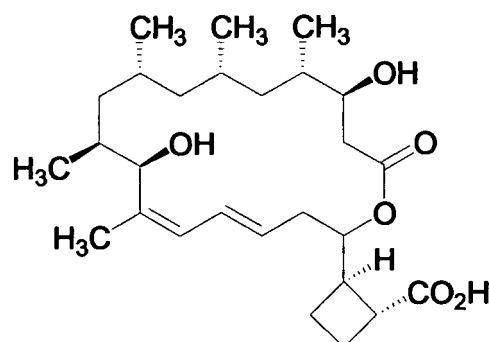
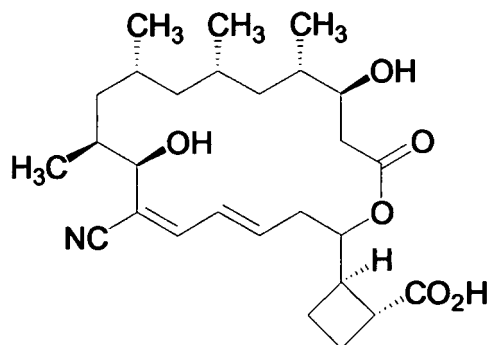


R₁ is a cycloalkyl group substituted as shown below;

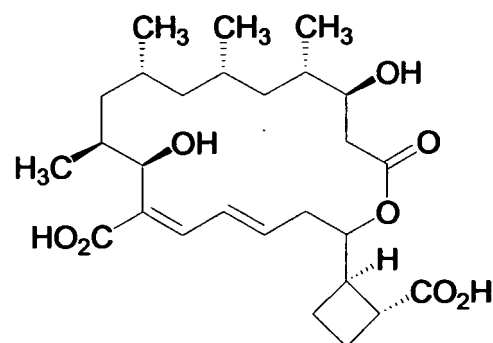


wherein R₁ can also optionally be substituted with one or more halo atoms or one or more C₁ to C₃ alkyl groups; R₂, R₃, R₆, or R₁₁ are each independently H, OCH₃, CH₃ or CH₂CH₃; and R₄ is CN, CO₂H or CH₃.

2. A compound or salt according to claim 1 wherein R₄ is CH₃ or COOH.
3. A compound or salt according to claim 1 wherein R₄ is CN.
4. A compound or salt according to claim 1 wherein R₁ is cyclobutane-1'-carboxylate.
5. A compound or salt according to claim 4, wherein R₄ is CH₃ or COOH.
6. A compound or salt according to claim 1, wherein R₆ is CH₃, R₂ and R₁₁ are H and R₁ is cyclobutane-1'-carboxylate.
7. A compound or salt thereof according to claim 1 wherein the compound is selected from the list consisting of:



and



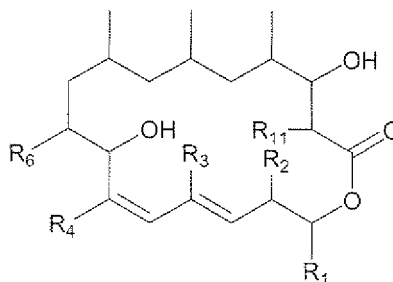
8. The use of a compound according to any one of claims 1 to 7 in the preparation of medicament for the treatment of microbial conditions including malaria, for the inhibition of angiogenesis, for the treatment of proliferative disorders, or for the treatment of conditions **characterised by** inappropriate vascularisation.
9. The use according to claim 8, wherein the medicament is an anti-cancer agent.
10. The use according to claim 8 wherein the proliferative disorder is psoriasis
11. The use according to claim 8 wherein the condition **characterized by** inappropriate vascularisation is selected from the group consisting of psoriasis, rheumatoid arthritis, atherosclerosis and diabetic retinopathy.
12. The use of a compound according to any one of claims 1 to 7 in the manufacture of a medicament for the inhibition of angiogenesis.
13. A compound according to any one of claims 1 to 7 for use as a medicament.

14. A pharmaceutical composition comprising a compound according to any one of claims 1 to 7 in admixture with a pharmaceutically acceptable excipient, carrier, buffer or stabiliser.
15. A compound according to any one of claims 1 to 7 for use as a medicament for the treatment of microbial conditions including malaria, for the inhibition of angiogenesis, for the treatment of proliferative disorders, or for the treatment of conditions **characterised by** inappropriate vascularisation.

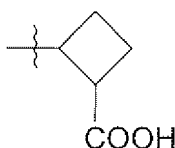
Patentansprüche

1. Verbindung der Formel 1 oder pharmazeutisch unbedenkliches Salz davon, wobei:

Formel 1

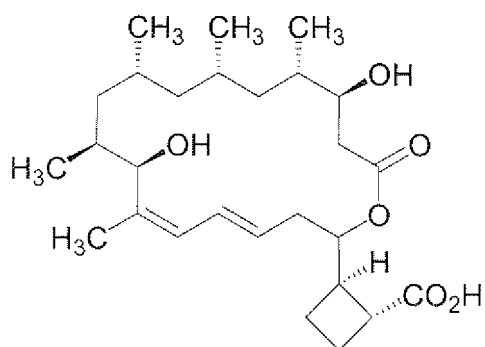
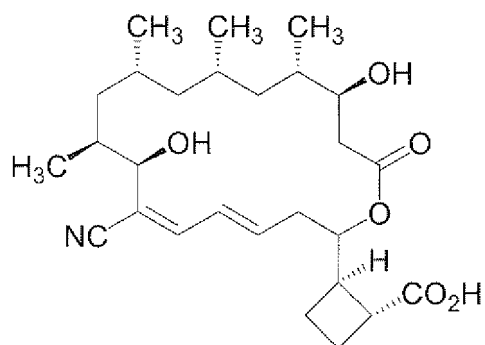


R₁ für eine wie nachfolgend gezeigt substituierte Cycloalkylgruppe steht,

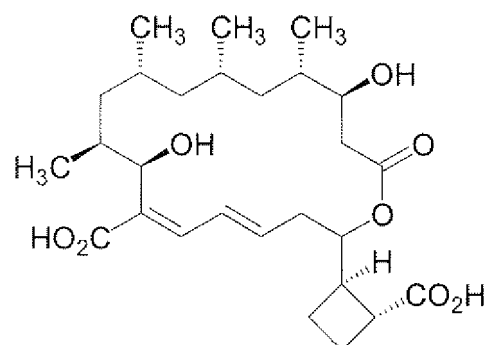


wobei R₁ gegebenenfalls auch mit einem oder mehreren Halogenatomen oder einer oder mehreren C₁- bis C₃-Alkylgruppen substituiert sein kann; R₂, R₃, R₆ oder R₁₁ jeweils unabhängig für H, OCH₃, CH₃ oder CH₂CH₃ stehen; und R₄ für CN, CO₂H oder CH₃ steht.

2. Verbindung oder Salz nach Anspruch 1, wobei R₄ für CH₃ oder COOH steht.
3. Verbindung oder Salz nach Anspruch 1, wobei R₄ für CN steht.
4. Verbindung oder Salz nach Anspruch 1, wobei R₁ für Cyclobutan-1'-carboxylat steht.
5. Verbindung oder Salz nach Anspruch 4, wobei R₄ für CH₃ oder COOH steht.
6. Verbindung oder Salz nach Anspruch 1, wobei R₆ für CH₃ steht, R₂ und R₁₁ für H stehen und R₁ für Cyclobutan-1'-carboxylat steht.
7. Verbindung oder Salz davon nach Anspruch 1, wobei die Verbindung ausgewählt ist aus der Liste bestehend aus:



und



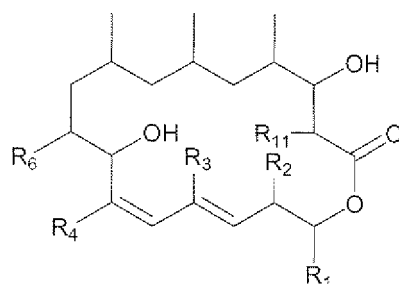
8. Verwendung einer Verbindung nach einem der Ansprüche 1 bis 7 bei der Herstellung eines Arzneimittels zur Behandlung mikrobieller Erkrankungen, einschließlich Malaria, zur Hemmung der Angiogenese, zur Behandlung proliferativer Störungen oder zur Behandlung von durch ungünstige Vaskularisierung gekennzeichneten Leiden.
9. Verwendung nach Anspruch 8, wobei es sich bei dem Arzneimittel um ein Antikrebsmittel handelt.
10. Verwendung nach Anspruch 8, wobei es sich bei der proliferativen Störung um Psoriasis handelt.
11. Verwendung nach Anspruch 8, wobei das durch ungünstige Vaskularisierung gekennzeichnete Leiden ausgewählt ist aus der Gruppe Psoriasis, rheumatoide Arthritis, Atherosklerose und diabetische Retinopathie.
12. Verwendung einer Verbindung nach einem der Ansprüche 1 bis 7 bei der Herstellung eines Arzneimittels zur Hemmung der Angiogenese.
13. Verbindung nach einem der Ansprüche 1 bis 7 zur Verwendung als Arzneimittel.

14. Pharmazeutische Zusammensetzung, umfassend eine Verbindung nach einem der Ansprüche 1 bis 7 im Gemisch mit einem pharmazeutisch unbedenklichen Hilfsstoff, Trägerstoff, Puffer oder Stabilisator.
15. Verbindung nach einem der Ansprüche 1 bis 7 zur Verwendung als Arzneimittel zur Behandlung mikrobieller Erkrankungen, einschließlich Malaria, zur Hemmung der Angiogenese, zur Behandlung proliferativer Störungen oder zur Behandlung von durch ungünstige Vaskularisierung gekennzeichneten Leiden.

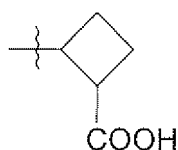
Revendications

1. Composé de formule 1 ou un sel pharmaceutiquement acceptable de celui-ci, dans laquelle :

formule 1 :

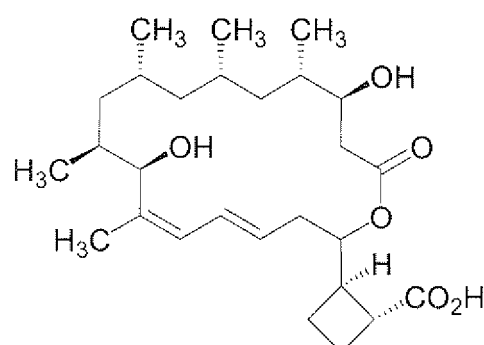
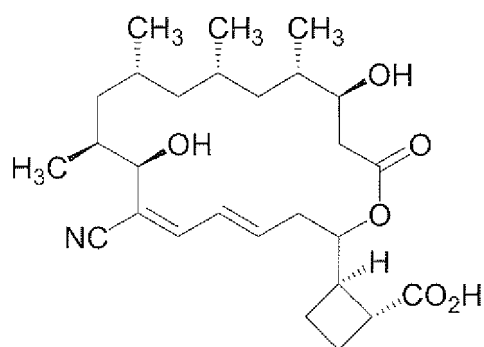


R_1 est un groupe cycloalkyle substitué comme montré ci-dessous ;

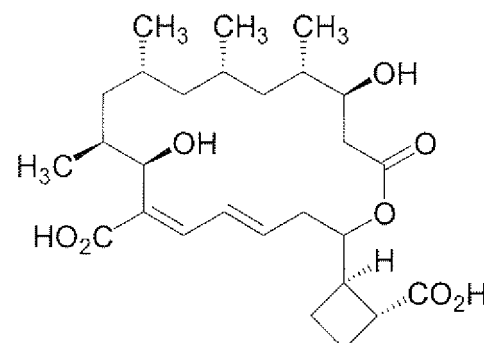


où R_1 peut éventuellement aussi être substitué par un ou plusieurs atomes d'halogène ou un ou plusieurs groupes alkyle en C_1 à C_3 ; R_2 , R_3 , R_6 , ou R_{11} représentent, chacun indépendamment, H, OCH_3 , CH_3 ou CH_2CH_3 ; et R_4 représente CN, CO_2H ou CH_3 .

2. Composé ou sel selon la revendication 1, où R_4 représente CH_3 ou $COOH$.
3. Composé ou sel selon la revendication 1, où R_4 représente CN.
4. Composé ou sel selon la revendication 1, où R_1 représente cyclobutane-1'-carboxylate.
5. Composé ou sel selon la revendication 4, où R_4 représente CH_3 ou $COOH$.
6. Composé ou sel selon la revendication 1, où R_6 représente CH_3 , R_2 et R_{11} représentent H et R_1 représente cyclobutane-1'-carboxylate.
7. Composé ou sel de celui-ci selon la revendication 1, où le composé est choisi dans la liste constituée par :



et



8. Utilisation d'un composé selon l'une quelconque des revendications 1 à 7 dans la préparation d'un médicament destiné au traitement d'états microbiens comprenant la malaria, à l'inhibition de l'angiogenèse, au traitement de troubles de prolifération ou au traitement d'états **caractérisés par** une vascularisation inappropriée.
9. Utilisation selon la revendication 8, où le médicament est un agent anticancéreux.
10. Utilisation selon la revendication 8, où le trouble de prolifération est le psoriasis.
11. Utilisation selon la revendication 8, où l'état **caractérisé par** la vascularisation inappropriée est choisi dans le groupe constitué par le psoriasis, l'arthrite rhumatoïde, l'athérosclérose et la rétinopathie diabétique.
12. Utilisation d'un composé selon l'une quelconque des revendications 1 à 7 dans la préparation d'un médicament destiné à l'inhibition de l'angiogenèse.
13. Composé selon l'une quelconque des revendications 1 à 7 destiné à une utilisation comme médicament.

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14. Composition pharmaceutique comprenant un composé selon l'une quelconque des revendications 1 à 7 en mélange avec un excipient, un support, un tampon ou un agent de stabilisation pharmaceutiquement acceptables.
- 5 15. Utilisation d'un composé selon l'une quelconque des revendications 1 à 7 destiné à une utilisation comme médicament destiné au traitement d'états microbiens comprenant la malaria, à l'inhibition de l'angiogenèse, au traitement de troubles de prolifération ou au traitement d'états **caractérisés par** une vascularisation inappropriée.

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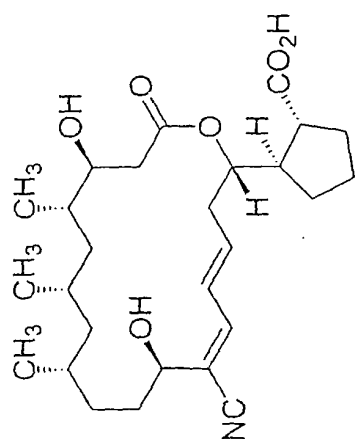
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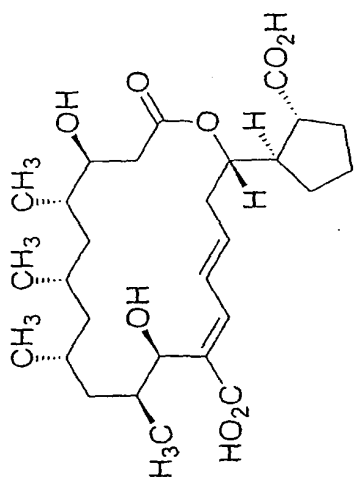
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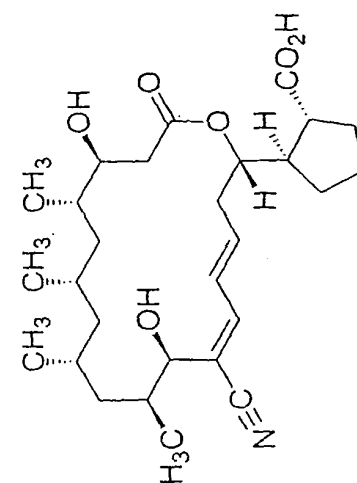
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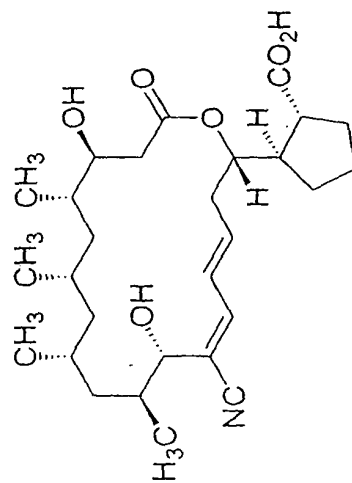
10-desmethyl borrelidin (3)



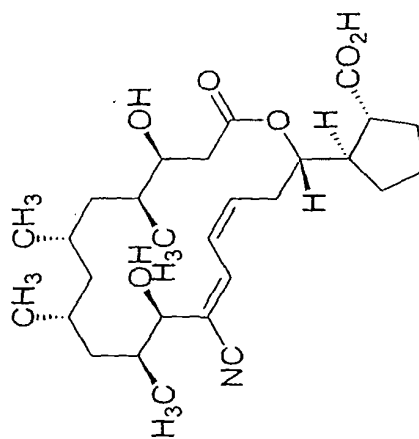
12-desnitrile-12-carboxyl borrelidin (2)



Borrelidin (1)



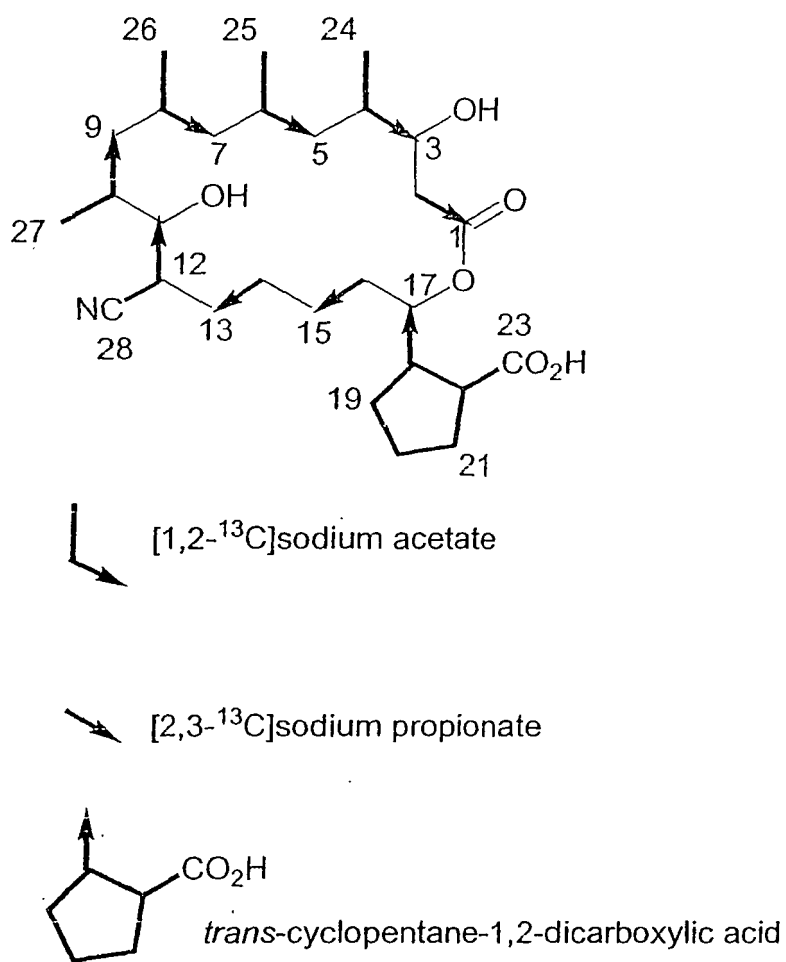
11-*epi* borrelidin (4)



14,15-*cis*-borrelidin (5)

Figure 1

Figure 2



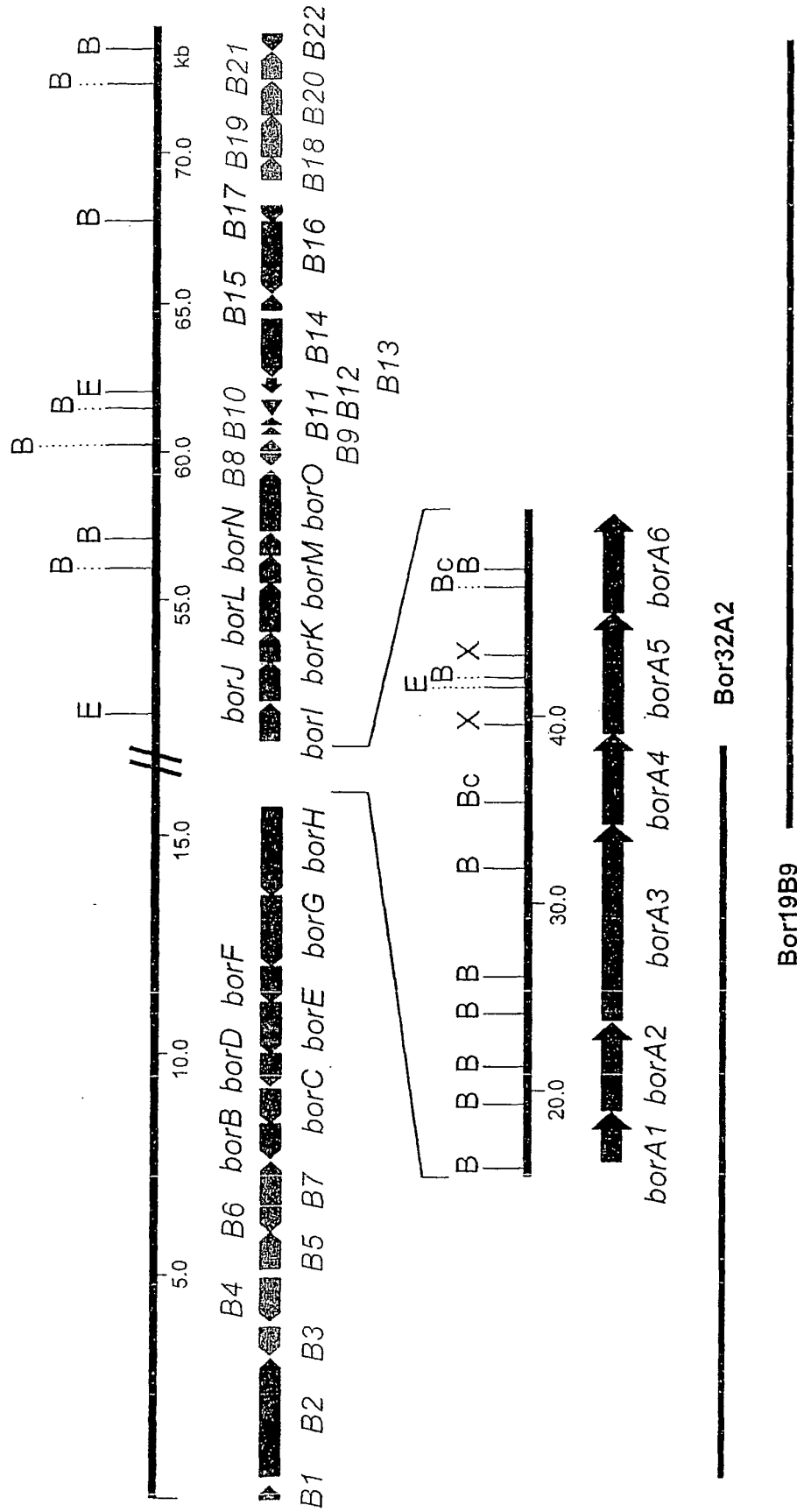


Figure 3

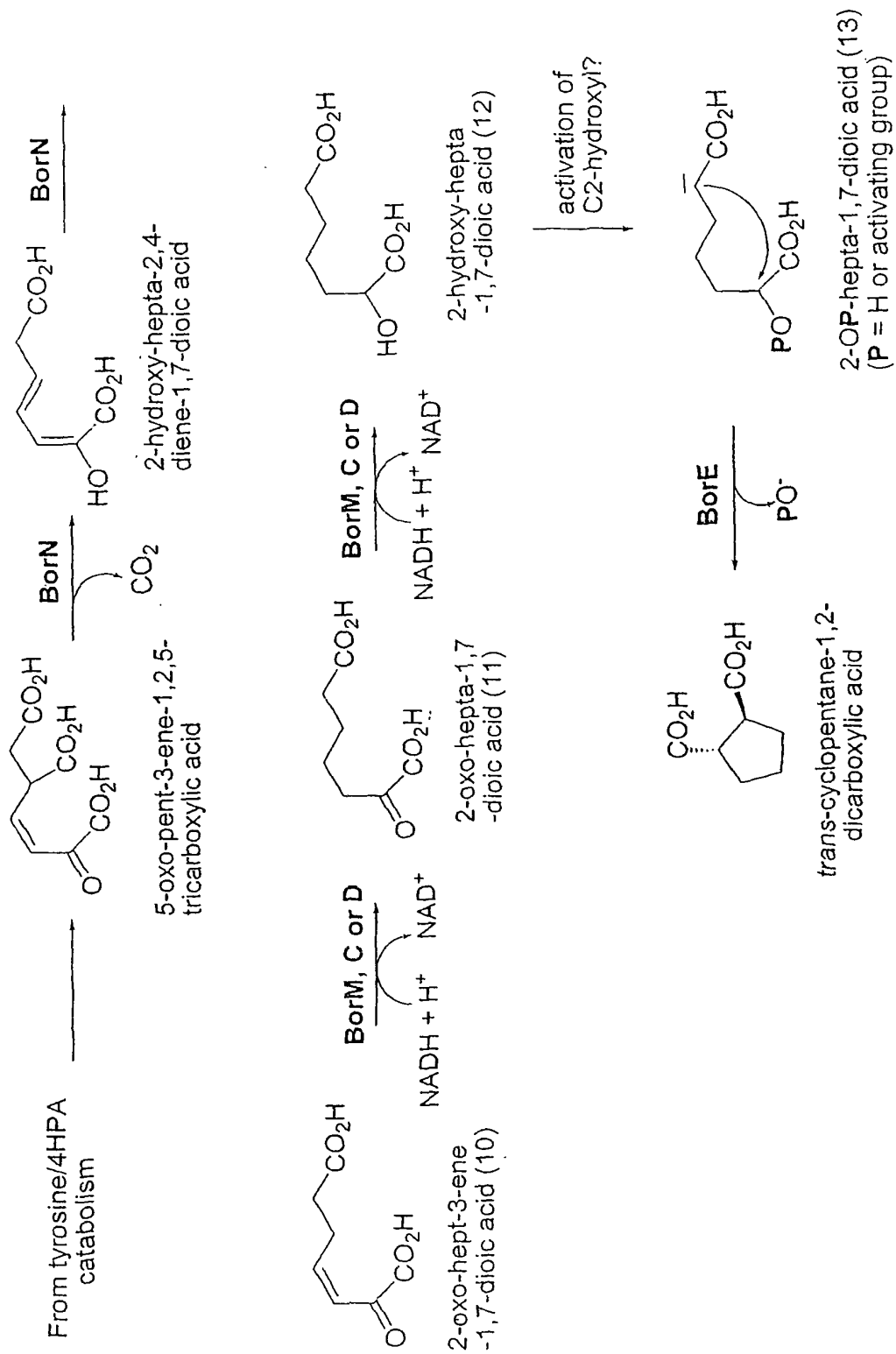


Figure 4

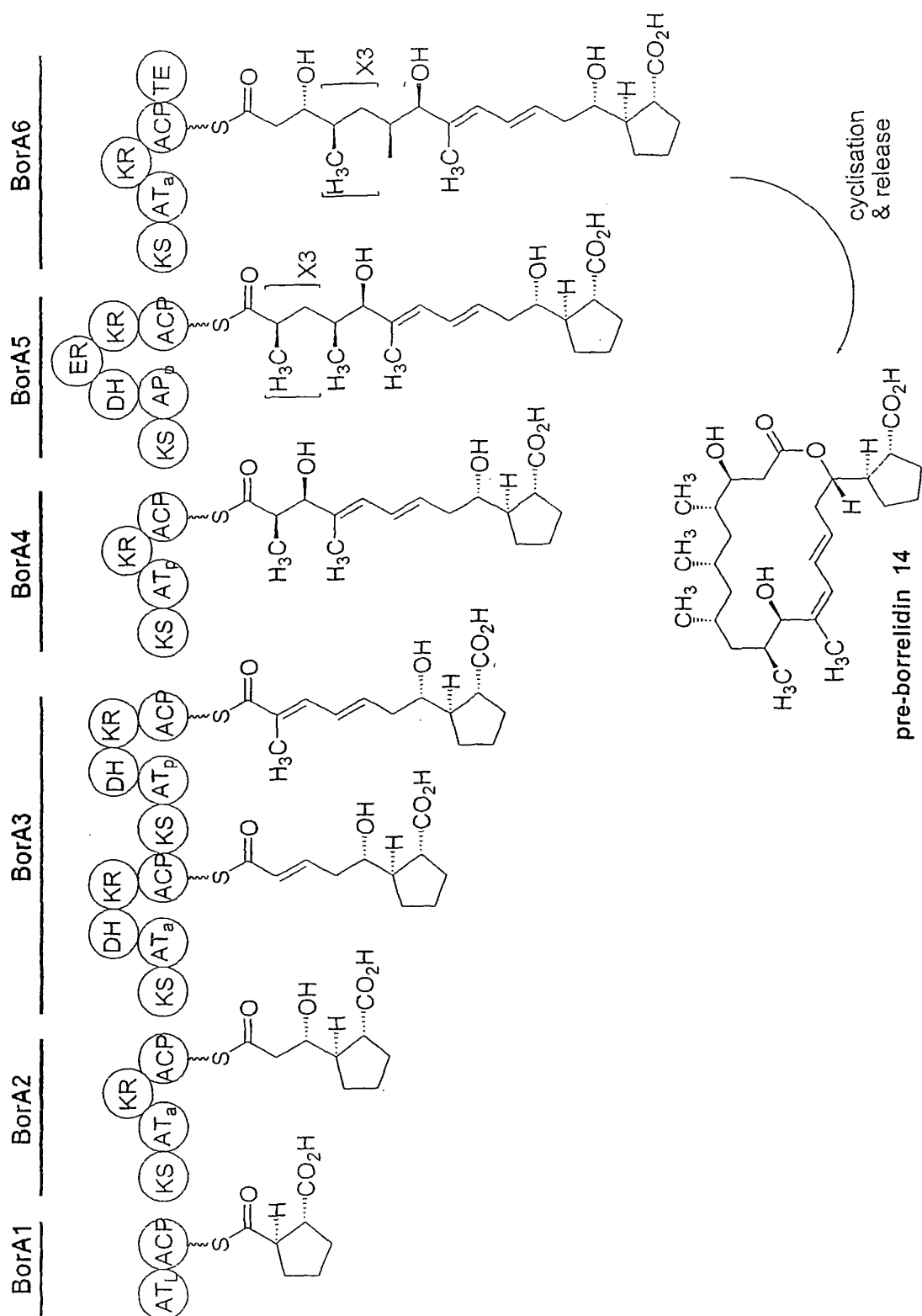


Figure 5

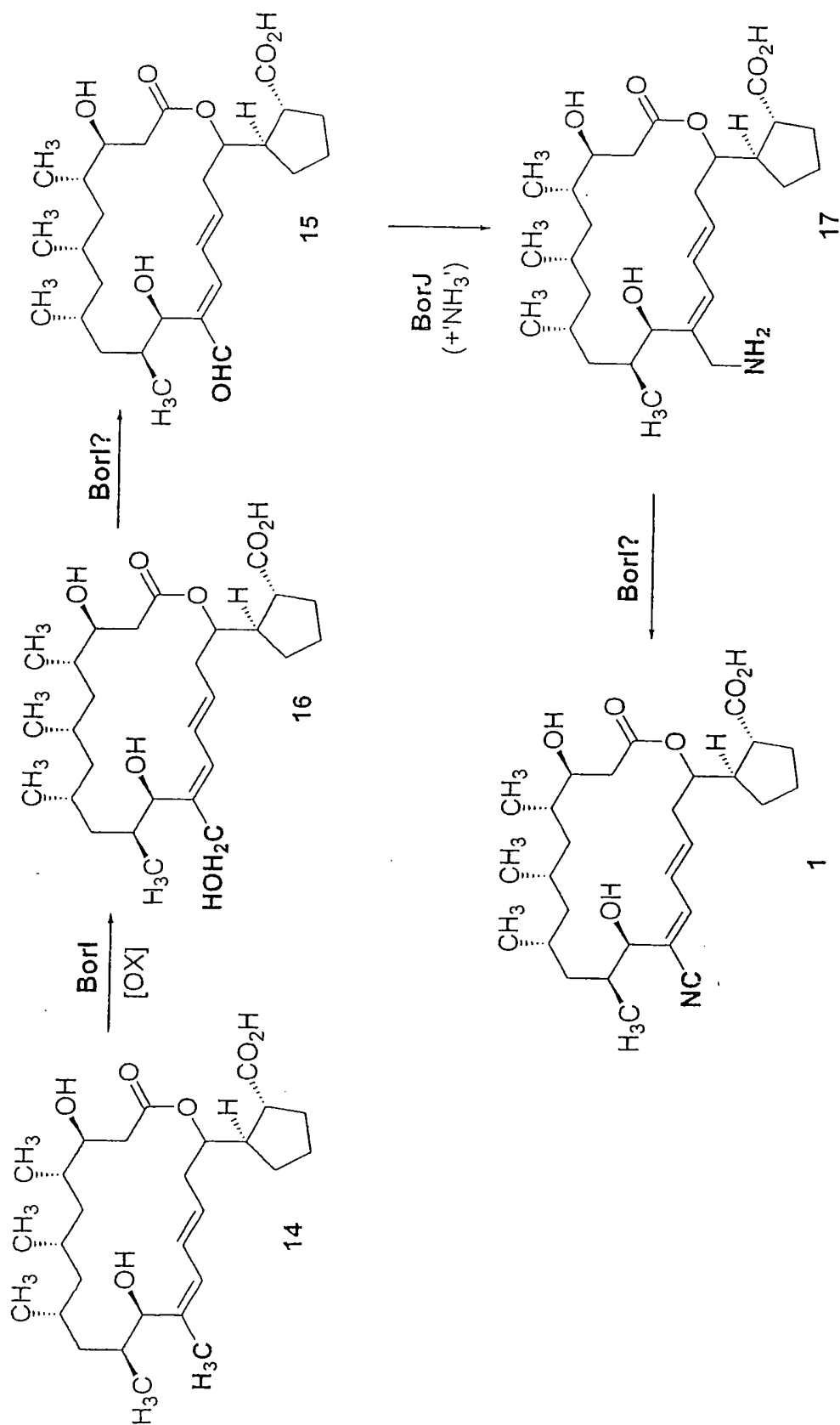
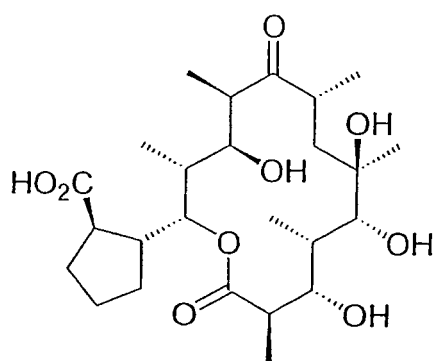


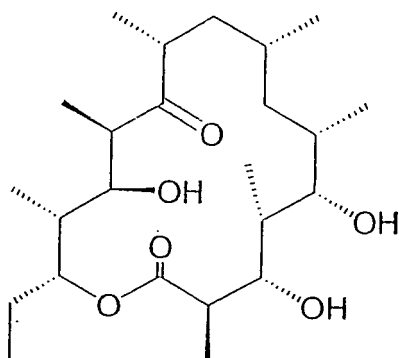
Figure 6

Figure 7

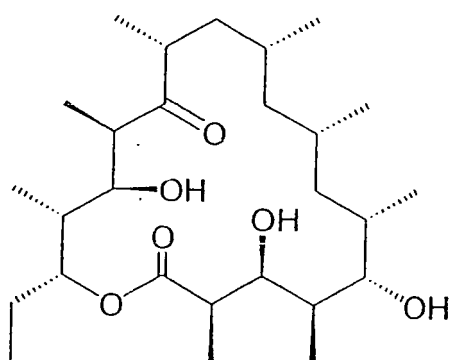


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Figure 8



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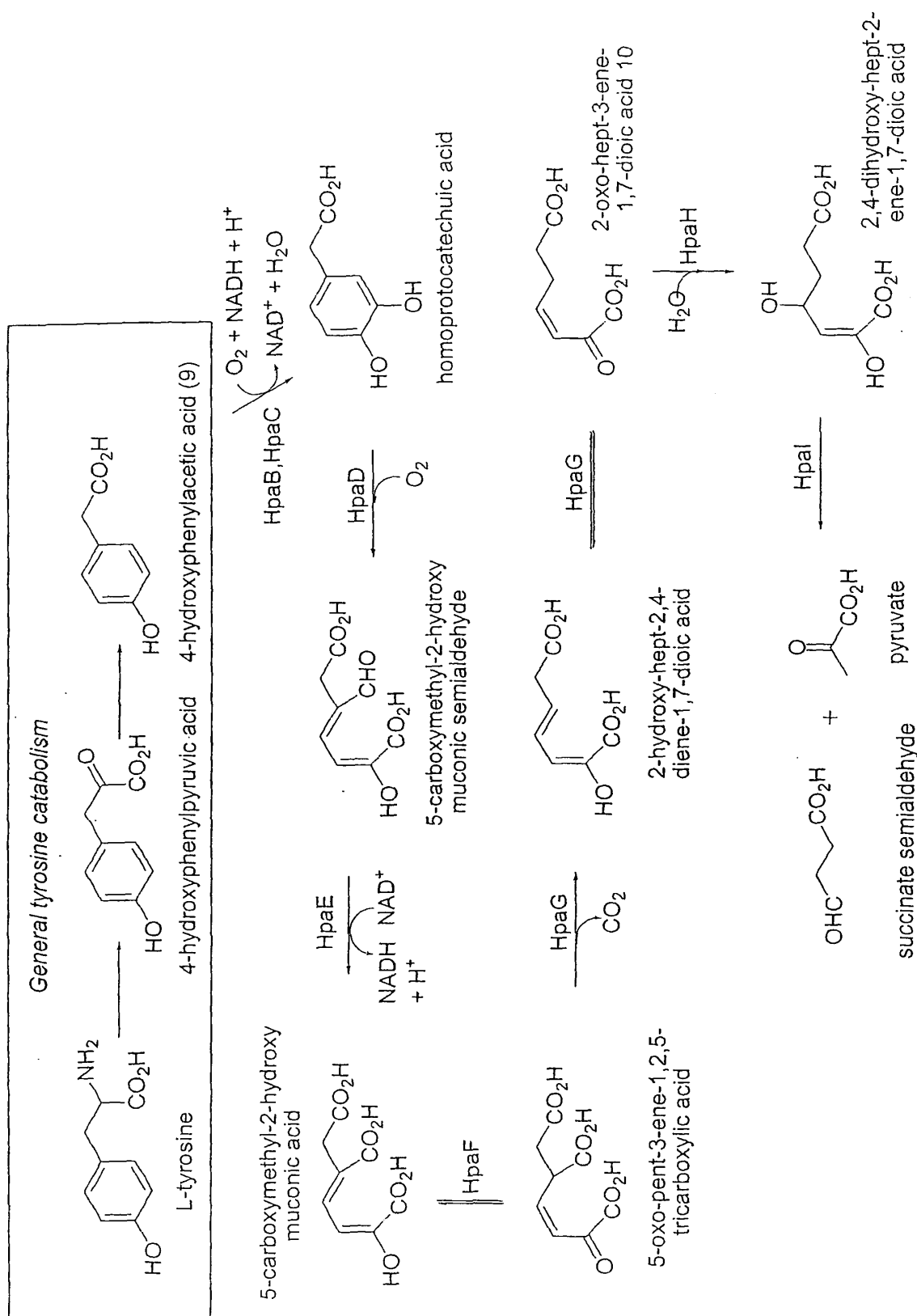
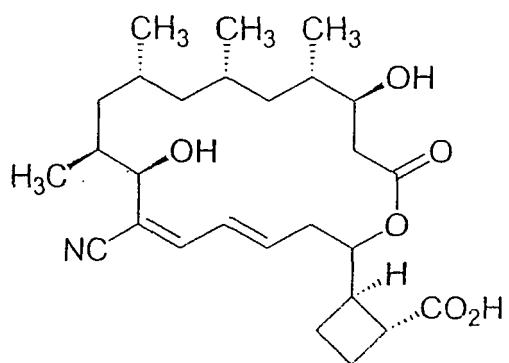
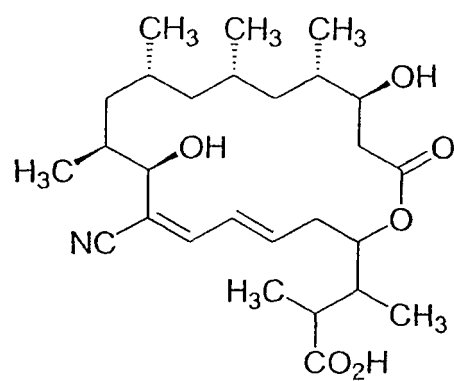


Figure 9

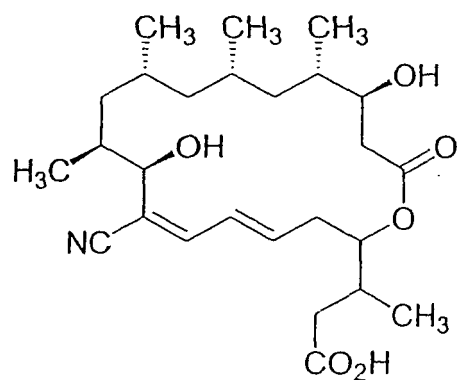
Figure 10



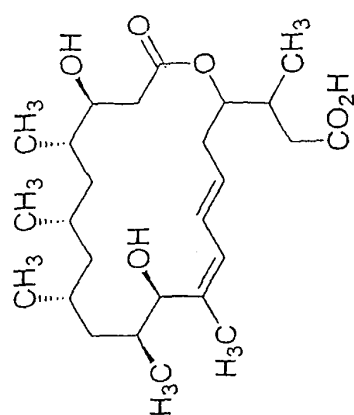
18: $m/z = 474.3$ ($[M-H]^-$)



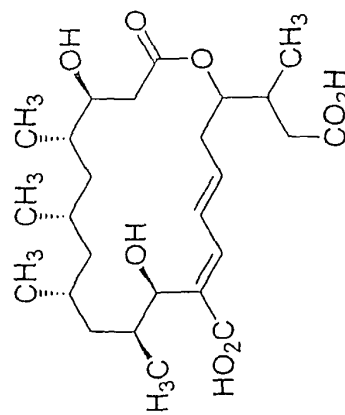
19: $m/z = 476.3$ ($[M-H]^-$)



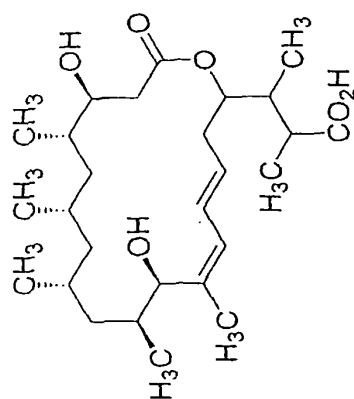
20: $m/z = 462.3$ ($[M-H]^-$)



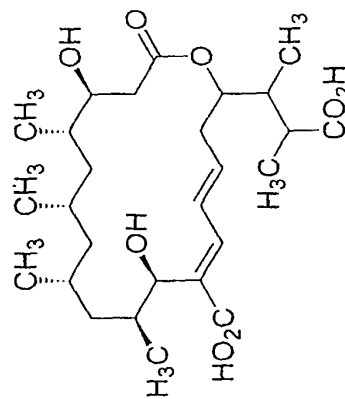
23: $m/z = 451.3$ ($[M-H]^+$)
 $\lambda_{\max} = 240$ nm



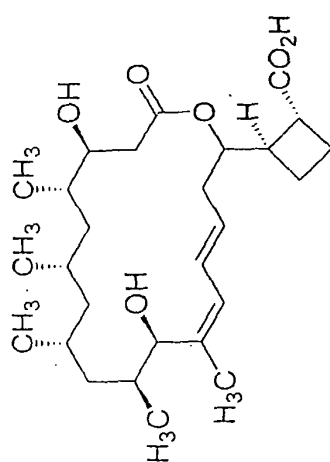
26: $m/z = 481.3$ ($[M-H]^+$)
 $\lambda_{\max} = 262$ nm



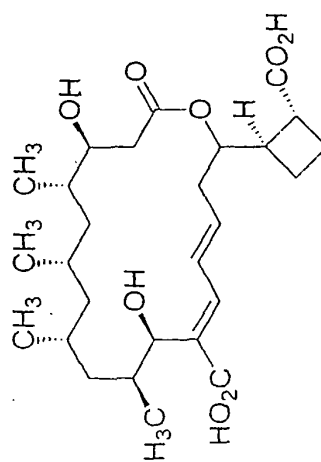
22: $m/z = 465.4$ ($[M-H]^+$)
 $\lambda_{\max} = 240$ nm



25: $m/z = 495.3$ ($[M-H]^+$)
 $\lambda_{\max} = 262$ nm



21: $m/z = 463.3$ ($[M-H]^+$)
 $\lambda_{\max} = 240$ nm



24: $m/z = 493.3$ ($[M-H]^+$)
 $\lambda_{\max} = 262$ nm

Figure 11